



THE AMERICAN JOURNAL  
OF PATHOLOGY





# THE AMERICAN JOURNAL OF PATHOLOGY

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## VOLUME VI

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# THE AMERICAN JOURNAL OF PATHOLOGY

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NUMBER I

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## DEGENERATIVE CHANGES IN THE MALE GERMINAL EPITHELIUM IN ACUTE ALCOHOLISM AND THEIR POSSIBLE RELATIONSHIP TO BLASTOPHTHORIA \*

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In a brief preliminary report <sup>1</sup> upon a small series of cases, and in a more extended abstract based upon a larger series,<sup>2</sup> I have summarized evidence in support of the belief that the changes in the human testis in acute alcoholism furnish a morphological basis for assuming for ethyl alcohol a blastophthoric effect upon human germ plasm. In the present paper this evidence is presented in detail for the first time, with a survey of the literature dealing with this method of approach to the question of alcoholic blastophthoria and a discussion of its implications.

The general acceptance by practically all of the pure biologists of the doctrine of the non-inheritance of acquired characteristics has had a deterrent effect upon the growth of our knowledge of alteration of the germ plasm through extrinsic factors. This has doubtless been due to the fact that changes in the germ plasm have been thought of as manifested particularly through the transmission or non-transmission of unit characters. Between such alterations which follow the recognized laws of genetics and the imposition of altered metabolism with resulting variation in potentiality for growth and differentiation there seems, at first, to be but little in common. In the future we may come to realize that essentially the same forces are operating in each type of change, crudely and diffusely in one, precisely and delicately in the other. The difficulty has been that with the biological conception of the non-

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inheritance of acquired characters emphasis has been placed upon the *stability* of the germ plasm. Actually, the germinal epithelium, particularly in certain stages of its maturation, is one of the most labile of all cell types, and it is the *lability* of the germ plasm which should be stressed.

Under the term *blastophthoria* may be grouped all of the processes of alteration, whether detrimental or beneficial, brought about in the germ plasm prior to amphimixis by extrinsic factors (in contrast to intrinsic germinal variation). A variety of chemical and physical agents are now known to be capable of exerting such an effect. It is obvious that it is in the male germ plasm particularly that blastophthoric processes can be recognized and studied, for postconceptional intra-uterine changes must be rigidly excluded. Moreover, much less is known about the normal and pathological histology of the ovary than of the testis.

There are three general methods by which injury to the male germinal epithelium may be investigated.

1. *The number and quality of the progeny* of supposedly altered germ plasm may be evaluated, a method much used in studies of human "race poisons." With human material the worth of the method may easily be overestimated. While presumptive evidence may be gathered in this manner, it is rare that results which can be considered proof can be obtained. The many interrelated variable factors make it impossible to develop such a statistical study in an entirely satisfactory manner. By adapting the same method to animal experimentation, however, the results may be greatly strengthened. By this method Stockard<sup>3</sup> established experimental proof of alcoholic blastophthoria and at the same time provided a model for the study of the blastotoxic effect of other agents.

2. *The number and quality (morphology, viability, vitality, motility, etc.) of the mature germ cells* may be compared after exposure to some extrinsic factor with the same attributes of germ cells under normal conditions. The exposure may be prior to maturation, after maturation but while yet within the parent body, or without the body but before fertilization has taken place. If variation from the normal can be demonstrated, it may be possible in experimental work to test the results of fertilization of, or by, such altered germ cells, in which case the method shifts to that first mentioned. In human material it is necessary to assume that cells so altered cannot

produce normal offspring. This assumption, however, is well supported by analogies from the field in which experimental test is possible.

3. *The processes of spermatogenesis and oögenesis* under the influence of extrinsic agents may be observed for alterations from the normal. As is well known, many agents when used intensively bring about complete aspermatogenesis through a process of degeneration of the germinal epithelium. It follows that in earlier stages of the process germ cells less seriously affected and still capable of fertilizing ova must have been produced. It must be realized that changes in the germ cells of such a nature as to give rise to markedly inferior offspring may be unrecognizable morphologically and that spermatozoa with demonstrable morphological changes may be incapable of fertilizing ova. That such marked changes are found as the end result of a continuous process of deviation from the normal justifies the use of this method, which is the one employed in the present study.

An abundant literature has accumulated in regard to the histological changes in the testis attributed to alcohol. This deals with both human and experimental material. In the former the earlier descriptions<sup>4</sup> were confused by the inclusion of changes now known to be due to syphilis, and it has rarely been possible to draw a sharp line between acute and chronic alcoholism in human material. It is interesting that the very frequent concomitance of syphilis and alcohol created the same difficulty in interpreting etiology in testicular pathology that still exists in regard to the liver. In experimental work these difficulties are avoided. The literature dealing with the testicular changes in both human and experimental alcoholism is briefly reviewed here in so far as it deals with relatively acute intoxication.

#### REVIEW OF LITERATURE

Bouin and Garnier,<sup>5</sup> in 1900, examined microscopically the testes of two rats to which they daily had administered progressively increasing doses of diluted alcohol. The exact amounts used were not stated. While the length of time over which the experiment extended ( $8\frac{1}{2}$  months in one instance and  $11\frac{1}{2}$  months in the other) was such as to make the word "acute" seem inappropriate, the results obtained were in accord with the pathological conception of

an acute, in contrast to a chronic, parenchymatous degeneration. Since the dosage was progressively increased it may be that the threshold of toxicity producing morphological change was not reached until late in the experiment. In one rat the testes appeared smaller and somewhat firmer than normal, in the other very soft. On section neither fibrosis nor vascular sclerosis was found. There was some diminution in the size of the tubules and varying degrees of reduction in the thickness of the germinal epithelium. There were but very few normal spermatozoa present but atypical free cells were found in the lumina. The disappearance of the seminal elements was found to be taking place in an order inverse to that of their origin. Pyknosis, chromatolysis and karyorrhexis of the nuclei; hyaline degeneration, plasmorrhexis and fatty change in the cytoplasm; vacuolar degeneration of both cytoplasm and nucleus, and the coalescence of spermatocytes or spermatids to form polynuclear plasmatic masses were described. They concluded that the seminal epithelium is highly vulnerable to prolonged ethyl alcohol intoxication, the more completely differentiated cells suffering more severely.

Todde,<sup>6</sup> in 1910, poisoned three cocks with large doses of ethyl alcohol. The first died in eight hours after the administration of about 50 cc. of twenty per cent ethyl alcohol. Its testes weighed 25 gm. and appeared of normal size and consistency. Of the second, which was killed after three successive daily intoxications, the testes were slightly smaller than normal, weighing 18 gm. The third was killed after four days and its testes were found to be much smaller than normal, weighing but 14.5 gm., although they showed nothing unusual in respect to color or consistency. Histological examination showed no marked changes. There was a little enlargement of the lumina of the tubules but "except for an indistinct and slight tendency to diffuseness all of the elements preserved a normal appearance and in most of the tubules, spermatogenesis was proceeding at its maximum intensity." It is difficult to reconcile these slight histological changes with an apparent reduction in weight to the extent of about 40 per cent as shown in the third instance.

Stockard and Craig,<sup>7</sup> likewise, failed to find changes of significance in the testes and ovaries of guinea pigs which had been treated with alcohol vapor for varying periods. In all cases the germ cells, both ova and spermatozoa, were found to exhibit an entirely normal structure.

Arlitt and Wells,<sup>4</sup> in 1917, made a detailed histological study of the testes of fifteen rats which had received from 0.25 to 2.25 cc. of ethyl alcohol daily for from two to ten months. Marked and nearly constant changes were found. A marked decrease in the size of the seminiferous tubules, leading in some instances to reduction in size of the testis itself, was shown by careful measurements with an ocular micrometer. The changes in the seminal elements appeared to take place in a definite order, inverse to that of the appearance of the various cell types. The first effect of the alcohol appeared to be to render the formation of spermatozoa incomplete, so that heads were formed without normal tails. This was further borne out by the fact that in such testes the number of spermatozoa in the epididymis was less than normal and proportionately less than the number seen in the seminiferous tubules. The next effect seemed to be to prevent the transformation of spermatids into spermatozoa, so that the tubules became filled with accumulated spermatids. The spermatids then degenerated, losing their power of nuclear staining and becoming granular. In the most advanced stages the tubules contained but marginal cells, with few or no spermatocytes or spermatids and occasional large cells with many nuclei free in the lumen. When the tubules were atrophic there was some compensatory edema *ex vacuo*. No inflammatory changes were seen and no marked fibrosis although in certain instances there had been a slight thickening of the basement membranes. The authors stated that the testicular changes found were in harmony with autopsy findings in human alcoholics. The ovaries of alcoholized females were examined also, but it was found impossible to make any positive statement as to alterations attributable to alcohol.

Kostitch,<sup>8</sup> in 1922, described the determination of an experimental alcoholic blastophthoria by histological study of the seminal epithelium. The testes of rats to which he had administered daily progressively increasing amounts of ethyl alcohol, from 0.5 to 3 cc. for from 17 to 120 days, were described in detail. Only his conclusions can be restated here: The seminal epithelium is especially sensitive to the action of alcohol. Its cells disappear in an inverse order to that of their genesis. At first the tubules become filled with masses of cells through desquamation of the more mature elements, with an accumulation of spermatids arrested in their development. Maturation divisions of the spermatocytes are disordered.

The accumulation of spermatids determines the formation of seminal teratocytes (teratospermatids). The arrest of spermatogenesis and the release of teratocytes reduces the epithelium to the primitive germ layer, the persistence of which makes possible a later regeneration. The sensitivity of the germinal epithelium to alcohol is much greater than that of the hepatic cells. Lesions of the germinal epithelium occur at a time when the hepatic cells show nothing. The lesions observed in the testis in the course of experimental alcoholic intoxication must be attributed directly to the action of pure ethyl alcohol. The nuclear changes in the germinal epithelium represent the essential phenomena of alcoholic blastophthoria. Short of being killed, the germinal epithelial cells exhibit alterations in their evolution. They may be arrested at any stage or may develop in an atypical manner leading especially to the formation of asymmetric mitoses with an unequal division of the chromosomes, a process in which there may be found an explanation of the occurrence of morphological defects in the children of alcoholics.

Apparently Kostitch was not familiar with the work of Arlitt and Wells, for he does not include it in his summary of the literature. The close agreement in the descriptions of the histological changes in these two detailed studies is significant.

## MATERIAL

Nine coroner's autopsies were selected in which death occurred during, or immediately after, a period of severe alcoholic intoxication. No case with a massive pneumonia or other extensive acute infectious condition was admitted to the series. In using human material, unlike that from animal experimentation, it is practically impossible to maintain criteria any more rigid than those stated above. Contrary to the impression given by the daily press in regard to certain other clinical centers, we have fewer autopsies by far on cases of alcoholism than we did prior to national prohibition.

There are three objections to the proposed utilization of this material which must be discussed. The first is pertinent and unavoidable. In most instances it cannot be asserted that the terminal bout of drinking was not an exacerbation of an intermittent or more or less chronic alcoholism. To this extent the intoxication may not be strictly acute. On the other hand, the changes which are found

are of an acute degenerative nature, entirely comparable to those found in experimental animals. Outside of a rather narrow range, the exact length of time that the condition of alcoholism obtains seems to make relatively little difference in the picture. In every case used there had been a severe degree of intoxication shortly before death.

The frequency with which areas of syphilitic orchitis occur in the testes of these cases may raise a suspicion that the other changes which are to be described were due to syphilis and not to alcohol. There is no connection, and no resemblance, between the two pathological processes and, to one experienced in histopathological diagnosis, no possibility of confusion exists. Exactly similar patches of orchitis fibrosa syphilitica occur in the testes of men dying from a variety of conditions without the remainder of the parenchyma showing the degenerative changes found in the present group. Syphilitic changes in the testes are patchy in distribution and may be unilateral. Blastotoxic degenerations, such as are attributed to alcohol, are diffuse and bilateral. The syphilitic changes are primarily vascular; the alcoholic changes are primarily parenchymatous.

It will be noted that the complete autopsy examination showed various incidental conditions to be present in certain cases, particularly in the older men. These varying conditions can scarcely be important factors in causing the degenerative changes found in the testicular epithelium, which are all of the same general type and, taken together, constitute a unified retrogressive process. Moreover, in other autopsies all of these incidental disease conditions have been observed without accompanying alteration in spermatogenesis. The assumption of a causal relationship for alcohol in respect to the testicular degenerations described in these cases seems, therefore, to be justified.

CASE I. A boy, aged 17 years, was found dead in his automobile in the early morning after a drinking bout lasting part of the night. He was known to have been a user of alcohol before that time. The autopsy diagnosis was as follows:

Acute alcoholism. Congestion and edema of brain with cloudy swelling of ganglion cells. Congestion and edema of meninges with minute petechial hemorrhages in brain and meninges. Severe acute degenerative catarrhal gastritis. Extreme congestion and edema of all organs. Acute fatty degenerative infiltration of heart and liver.

Acute lipoidosis of adrenals. Acute cloudy swelling of kidneys. Lymphatic constitution.

Microscopic examination of the *testes* showed tubules which appeared unusually large, the lumina being dilated without thinning of the wall. On the contrary there was a moderate, but definite increase in the number of spermatocytes and spermatids present. Apparently normal spermatozoa were found in most of the tubules. The appearances suggested a retardation in the evolution of the various orders of cells of such a nature that the total number of spermatocytes and spermatids present at one time was increased. Numerous division figures occurred some of which may have been atypical although this cannot be positively asserted. No teratocytes or giant nuclear forms were seen.

CASE 2. A laborer, aged 42 years, was said to have consumed an enormous quantity of homemade wine, fortified with some form of "bitters" or "wine of beef and iron," during the five days before his death. There had been severe diarrhoea and vomiting and he was said to have fallen downstairs once during the period of his intoxication. The final pathological diagnosis was:

Acute exacerbation of chronic alcoholism. Chronic leptomeningitis. Acute encephalitis. Atrophy, passive congestion and edema of brain. Acute toxic gastro-enteritis on an older chronic catarrhal inflammation. Chronic esophagitis. Fatty heart. Polypoid thrombus in right ventricle. Atherosclerosis. Chronic passive congestion of lungs and acute purulent bronchopneumonia (aspiration pneumonia). Chronic parenchymatous degenerative nephritis. Fatty degenerative infiltration of liver. Acute purulent prostatitis with multiple abscesses. Healed tuberculosis of apices and spleen. Traumatic abrasions of skin. Hypertrophy of adrenals. Atypical spermatogenesis.

In addition to the evidences of acute and chronic alcoholism various incidental findings were encountered in this instance. The terminal aspiration pneumonia involved but small areas in the lower portion of each lung. It seems improbable that it could have been responsible for the testicular change. The nephritis was old and of a very moderate degree. The cardiac failure was an end phase of the alcoholic bout. It seems proper, therefore, to attribute the acute degenerative changes primarily to alcohol.

Microscopically, the *testes* showed as the most striking feature an accumulation of spermatocytes and spermatids of so marked a de-

gree as nearly to fill the lumina of the tubules. These cells were not free but still in position in the epithelium. In areas the picture was that of solid cords of cells. A few normal appearing sperm cells were present in most of the tubules. An occasional giant nucleus was found in the zone of spermatogonia, but no free teratocytes. Vacuolar degeneration was lacking. There was a very slight diffuse thickening of the basement membranes.

CASE 3. A farmer, about 50 years old, sold a load of produce, bought and drank a large quantity of whisky and was put upon his wagon while drunk by his companions. When his team of horses reached home he was found dead in the wagon. The pathological diagnosis was as follows:

Acute alcoholism. Congestion and edema of brain. Fatty degenerative infiltration of heart and liver. Acute congestion of all organs. Chronic catarrhal gastro-enteritis. Early sclerosis. Prostatic hyperplasia.

Microscopic examination of the *testes* showed a few spermatozoa in many tubules. The lumina of the tubules were dilated and the epithelium of very uneven thickness, in many areas being definitely reduced. Small masses of desquamated spermatids, apparently agglutinated, were found in many tubules. Of the spermatogonia and spermatocytes some showed an early vacuolar degeneration of the cytoplasm. Occasional cells, apparently spermatogonia, with giant nuclei up to three times the normal diameter in size, were found resting upon the basement membrane. No free teratocytes were noted. There was no increase in the stroma.

CASE 4. A brick mason, 62 years old, was found dead in his room. The coroner's diagnosis was "acute alcoholism." The final pathological diagnosis was:

Acute exacerbation of chronic alcoholism. Chronic leptomeningitis. Congestion and edema of brain. Cloudy swelling of ganglion cells. Chronic atrophic catarrhal gastritis, alcoholic. Old syphilis. Chronic fibroid myocarditis. Brown atrophy of heart. Early sclerosis of aorta. Chronic passive congestion of lungs with stasis, edema and anthracosis. Healed tubercles in lungs with calcareous tubercles in bronchial nodes. Chronic interstitial pancreatitis. Lipoidosis of adrenals. Chronic passive congestion and atrophy of all organs. Atypical spermatogenesis. Areas of interstitial fibrosis in testes. Rhabdomyoma of kidney.



The changes incident to advancing years and an old latent syphilis were found in this instance in addition to evidences of alcoholism. The acute degenerative changes shown microscopically in the testes could not be due to either of the former. The teratoid neoplasm in the kidney was of small size and of no clinical importance.

The *testes*, upon microscopic examination, showed a few spermatozoa in many tubules. There were also numerous masses of free cells, chiefly spermatids and spermatocytes which were apparently agglutinated. The tubules were dilated, the size of the lumina being increased in part by the marked thinning of the epithelium which was frequently found reduced to two or three layers of cells. In selected tubules there was noted a well marked vacuolar degeneration of the epithelium. This change showed a zonal distribution involving particularly the spermatocytes with a zone of nearly normal-appearing spermatids internal to the region of vacuolation. Among the free cells in the lumina teratocytes occurred in small numbers. These were chiefly multinucleate masses with abundant pink-staining cytoplasm containing from four to eight or more nuclei of spermatid or even sperm-head type. These masses gave the impression of having been formed by nuclear divisions unaccompanied by division of the cytoplasm. Cells of spermatocyte type with two or four nuclei were found in small numbers embedded in the wall. Giant nuclei were numerous. There was a slight diffuse increase in thickness of basement membranes and a few small groups of tubules showing complete fibroid obliteration of syphilitic type.

CASE 5. A mulatto porter, aged 33 years, who was known to have been intoxicated for two days, was found dead in a toilet-room. The coroner's diagnosis was "acute alcoholism." The autopsy diagnosis was as follows:

Acute alcoholism. Marked acute catarrhal gastro-enteritis. Congestion, edema and parenchymatous degeneration of all organs. Multiple hemorrhages in lungs. Syphilis (leptomeningitis, active aortitis, adrenalitis, orchitis). Persistent thymus. General lymphoid hyperplasia. Small esophageal papilloma.

Microscopic examination of the *testes* showed a greatly reduced number of spermatozoa, but a few normal appearing sperm cells were found in many tubules. There was extensive desquamation of both spermatids and spermatocytes with masses of agglutinated cells blocking the lumina of certain tubules. The epithelium was

correspondingly thinned and showed a moderate vacuolar change with no sharp zonal localization. The only teratocytes found were deep in the epithelium, near the basement membrane, and consisted of large cells with three to four nuclei of spermatocyte type. A few cells with single giant nuclei were noted also. There was no general fibrosis, but a few small patches of fibroid atrophy of syphilitic type.

CASE 6. A laborer, whose apparent age was about 55 years, was found dead. The stomach contents had still a strong odor of whisky at the time of autopsy. The pathological diagnosis was as follows:

Acute exacerbation of chronic alcoholism. Chronic syphilitic myocarditis, aortitis, pancreatitis and orchitis. General arteriosclerosis. Atrophy, passive congestion and parenchymatous degeneration of all organs. Early hepatitis. Lipoidosis of adrenals. Asphyxia. Hypernephroma.

The *testes* showed microscopically numerous apparently normal spermatozoa. The tubules were slightly dilated and in certain regions the number of spermatocytes and spermatids was increased to a very slight degree. Occasional tubules showed a rather sharply localized vacuolar change. A few teratocytes were found and only a few nuclei which definitely exceeded the normal range in size. There was a slight diffuse thickening of the basement membranes but no fibrosis of syphilitic type.

CASE 7. A young man who appeared to be about 30 years old, was placed under arrest while in an active alcoholic delirium. Chloral hydrate was administered to quiet him, the drug being given in more than ordinary therapeutic amount. He was found dead in his cell the following morning. The pathological diagnosis was as follows:

Acute alcoholism. Chloral hydrate poisoning. Passive congestion and stasis of all organs. Edema of lungs. Chromatolysis of cortical cells. Lipoidosis of adrenals. Early sclerosis of aorta. Excessive hemolysis.

Microscopic examination of the *testes* showed very few spermatozoa. The lumina of the tubules appeared dilated, largely because of the thinning of the epithelium. There was a well marked vacuolar change in both cytoplasm and nuclei, especially the latter. In certain tubules the vacuolar change occupied an intermediate zone and in a few the apparent transposition of the order of spermatogenetic cells, noted by Kostitch, was seen. In these areas a row of sper-

matids was found external to a zone of spermatocytes of the second order. Apparently these spermatocytes had suffered an arrest of development while the epithelium beneath them had brought on a new generation of spermatids. Free teratocytes, masses of cytoplasm with multiple nuclei, were found in small numbers. Usually two or four nuclei were present in each; in one, twelve could be counted. There were a few giant nuclei. There was no increase in the stroma.

The possibility that chloral hydrate poisoning was a factor in producing the changes found cannot be entirely excluded. It will be recognized, however, that the deviation from the normal is more marked than in any of the previously described cases. Not more than five or six hours had elapsed between the administration of this drug and death. It is impossible that the changes found could have been produced entirely within that interval, although they may have been intensified.

CASE 8. A farmer, formerly a miner, had been drinking heavily for some time and was intoxicated during the day and evening preceding his death. He went to sleep in a barn with a companion and was found dead the next morning. The autopsy yielded the following diagnosis:

Homicide. Fracture of intervertebral disc between sixth and seventh cervical vertebrae. Laceration of cord. Fresh traumatic ecchymoses of skin and cervical tissues with massive, fresh, retropharyngeal hematoma. Hemorrhagic suffusion of all mediastinal tissues. Death by asphyxia. Pulmonary stasis, edema and petechial hemorrhages. Old healed tuberculosis of lungs and of bronchial, mediastinal, mesenteric and retroperitoneal lymph nodes. Old adhesive pleuritis. Coronary sclerosis. Atrophy of myocardium. Epicardial sclerosis. Atherosclerosis of aorta. Chronic catarrhal gastro-enteritis. Acute exacerbation of chronic alcoholism. Atrophy, acute passive congestion and parenchymatous degeneration of all organs. Obliterated appendix. Old vesiculitis. Glandular hyperplasia of prostate. Old infarcts in renal cortex. Hypertrophic spondylitis of the lower thoracic vertebrae. Lipoma of perineum.

Death must have occurred in a relatively short time after injury to the cervical vertebrae and spinal cord. The changes described for the testes were of an acute zonal character and could not have been produced by the asphyxia resulting from that injury.

Microscopically the *testes* showed active spermatogenesis with normal appearing spermatozoa in many tubules. The lumina of the tubules were somewhat dilated and contained many desquamated spermatids and spermatocytes both singly and in clumps. A few multinucleate forms were included among these. The greatly thinned epithelium showed a well marked vacuolar change. Giant nuclei in small numbers were noted. In addition to a slight diffuse thickening of the basement membrane there were patches of fibrosis of the syphilitic type.

CASE 9. A man, aged 44 years, was found unconscious in his bed. He was taken to the hospital, but died within twenty-four hours without regaining consciousness. The reflexes were unaltered and there was no paralysis. There were no marks of violence upon the body. Aside from the coma, frequent diarrheic stools were the only symptom. The diagnosis as determined at autopsy was as follows:

Acute exacerbation of chronic alcoholism. Beer drinker's liver, early stage. Chronic parenchymatous nephritis of moderate degree. General sclerosis. Acute and chronic passive congestion of all organs. General atrophy. Lipoidosis of adrenals. Syphilitic atrophy of testes. Chronic leptomeningitis.

Upon microscopic examination the *testes* showed almost complete aspermatogenesis. In only two tubules were normal-appearing spermatozoa found. There were but few free cells and the greatly thinned epithelium showed a marked vacuolar change. In certain tubules this was zonal in position, affecting spermatocytes most severely while a single row of spermatids near the lumen showed but little change. Neither teratocytes of the multinucleate form nor giant nuclei were found. The changes in the germinal epithelium were the most marked of any in the series. Numerous patches of old syphilitic fibrosis and a slight diffuse thickening of the basement membranes occurred.

In Table I the chief histological changes in the testes of these nine cases are summarized. The cases have been described above in an order which was determined in so far as possible by the duration of the acute alcoholism. In some instances relatively little was known in regard to this point, but the history and the general autopsy findings have been taken together somewhat arbitrarily to determine this order. In Table I, however, the order has been determined by the stage and degree of severity of the testicular changes alone.

TABLE I

*Summary of Microscopic Findings with Cases Arranged in Order of Increasing Severity of the Degenerative Process*

	CASE NUMBERS								
	1	2	6	3	4	5	8	7	9
Normal appearing spermatozoa present	++	+	++	+	+	+	+	±	±
Lumen occupied by masses of free cells	-	-	-	+	+	+	+	-	-
Tubules dilated . . . . .	+	+	+	+	+	-	±	+	±
Epithelium thinned . . . . .	-	-	-	+	+	+	+	+	+
Spermatids increased . . . . .	+	++	±	-	-	-	-	-	-
Spermatocytes increased . . . . .	+	++	±	-	-	-	-	-	-
Vacuolar degeneration of cytoplasm . .	-	-	±	+	+	+	+	+	++
Vacuolar degeneration of nuclei . . .	-	-	-	-	±	-	-	+	+
Zonal degenerative changes. . . . .	-	-	±	-	+	-	-	±	+
Teratocytes . . . . .	-	-	-	-	+	+	±	+	-
Giant nuclei . . . . .	-	+	±	+	+	+	+	±	-
Diffuse fibrosis of basement membrane.	-	±	±	-	±	-	+	-	+
Fibrosis of syphilitic type . . . . .	-	-	-	-	+	+	+	-	+

Some degree of parallelism exists between the two arrangements. This might have been closer if more accurate histories had been available.

*Spermatozoa:* Normal appearing spermatozoa were found in every case, although they were very few in two instances. This material had been fixed and stained by routine methods only (chiefly formol fixation with hemalum and eosin staining) so that finer morphological deviations could not be adequately investigated. Judgment had to be based very largely upon nuclear form and chromatin content. In spite of the marked degenerative changes present spermatogenesis had taken place recently or was still taking place in every instance. This supports the belief that the processes here described

are essentially acute in nature, and leaves open the possibility of impregnation during the stages of degeneration represented in this group.

*Increase in Spermatids and Spermatocytes:* In the first, and to a more marked degree in the second case, the increase in spermatids and spermatocytes was striking. Many tubules appeared as solid masses of cells, the lumen having been encroached upon so much as to be practically obliterated. This apparent retardation of spermatogenesis with resulting accumulation of immature forms occurred only when evidences of more severe degenerative changes were lacking. It must be considered one of the earlier manifestations of this type of injury.

*Desquamation and Reduction of the Epithelium:* With somewhat more severe injury extensive desquamation of the inner layers of the thickened epithelium occurred and free spermatids and spermatocytes as well as clumped aggregates appeared in the lumina. With this process vacuolation of the remaining epithelium was usually present. The epithelium was reduced to two or three layers of cells in those tubules with marked desquamation. This produced the appearance of dilatation of the tubules themselves due to the wide lumina. While no accurate measurements were attempted because of lack of standardization in fixation it was evident that the tubules themselves were actually somewhat smaller than normal.

*Vacuolar Degeneration:* Vacuolar degeneration of the cytoplasm was found in all of the more severely injured testes. This vacuolation is hydropic in nature and is part of a process leading in its most advanced stages to liquefaction necrosis. In three instances small vacuoles were found within the nuclei also. An interesting picture was presented by the four cases in which the vacuolation occurred with a zonal distribution, affecting spermatocytes or spermatogonia more severely than the spermatids nearer the lumen.

*Atypical Forms:* Without special methods the more minute deviations from normal in karyokinesis cannot safely be described. That such must have been present was shown by the grosser variations in morphology which were constantly encountered when degenerative changes were marked. These were chiefly of two sorts. In the basal layer hyperchromatic cells, some with enormous single nuclei which have been called "giant" nuclei were frequently found. In the lumen, and also still attached to the wall, multinucleate

"teratocytes" were frequently encountered. These had the appearance of being formed by repeated nuclear divisions without corresponding fission of the cytoplasm. Especially was it true of those with many nuclei that the amount of cytoplasm present was insufficient to sustain the belief that the teratocyte had been formed by the fusion of previously separate units.

*Fibrosis:* In four of the nine cases patches of fibrous orchitis of syphilitic type were found. It has previously been pointed out that this condition has nothing in common with the changes which are attributed to alcohol and cannot be confused with them. In three instances there was a slight, and in two a moderate, diffuse fibrosis of the basement membranes. It is possible that there may be a relationship between this and chronic alcoholism but at present there is no evidence by which this can be either affirmed or denied.

*Lack of Specificity:* The changes which are here attributed to acute alcoholism are in no sense specific for that condition. There are apparently many extrinsic factors which can produce similar degenerative processes. We have been able to duplicate every aspect of them in experimental lead poisoning in guinea pigs, and Mills<sup>9</sup> has described similar changes associated with pneumonia. Neither is there proof that alcohol acts directly upon the germinal epithelium. The immediate damage may be elsewhere, perhaps in the liver, and the testicular effect secondary.

*Blastophthoric Significance:* The significance of such a marked degenerative process as was found in the testes of these cases of acute alcoholism is evident. The changes described exceed in degree those which are produced in the course of the experimental demonstration of alcoholic blastophthoria by means of breeding experiments. It is certain, in view of the atypical cell forms and the marked degeneration of both cytoplasm and nuclei, that abnormal spermatozoa are produced. This demonstrates, therefore, a morphological basis for experimental alcoholic blastophthoria, for prior to the development of such marked degenerative changes, spermatozoa capable of giving rise to defective offspring must be set free. Procreation during a period of intoxication thus entails a definite hazard as to the quality of the offspring which may result. There is experimental evidence to show that the basal layer of cells in the tubular epithelium (primitive germinal epithelium) suffers the least and is capable of regenerating a new series of spermatogenetic cells.

## SUMMARY

In the testes of nine men who died in a period of acute alcoholism, parenchymatous degeneration leading, when sufficiently marked, to practically complete aspermatogenesis was found. In the testes showing less severe changes there was an increase in spermatocytes and spermatids with a decrease in spermatozoa. As a more severe change this was followed by desquamation releasing free cells and masses of cells in the lumina of the tubules. Vacuolation of the cytoplasm, sometimes zonal in character, and in severe cases vacuolation of the nuclei, occurred. The vacuolated epithelium became reduced to but one to four layers of cells, only the basal layer being intact. Atypical cell divisions producing hyperchromatic giant nuclei and multinucleate teratocytes were numerous.

These observations place clinically recognized, and experimentally induced, alcoholic blastophthoria upon a morphological basis.

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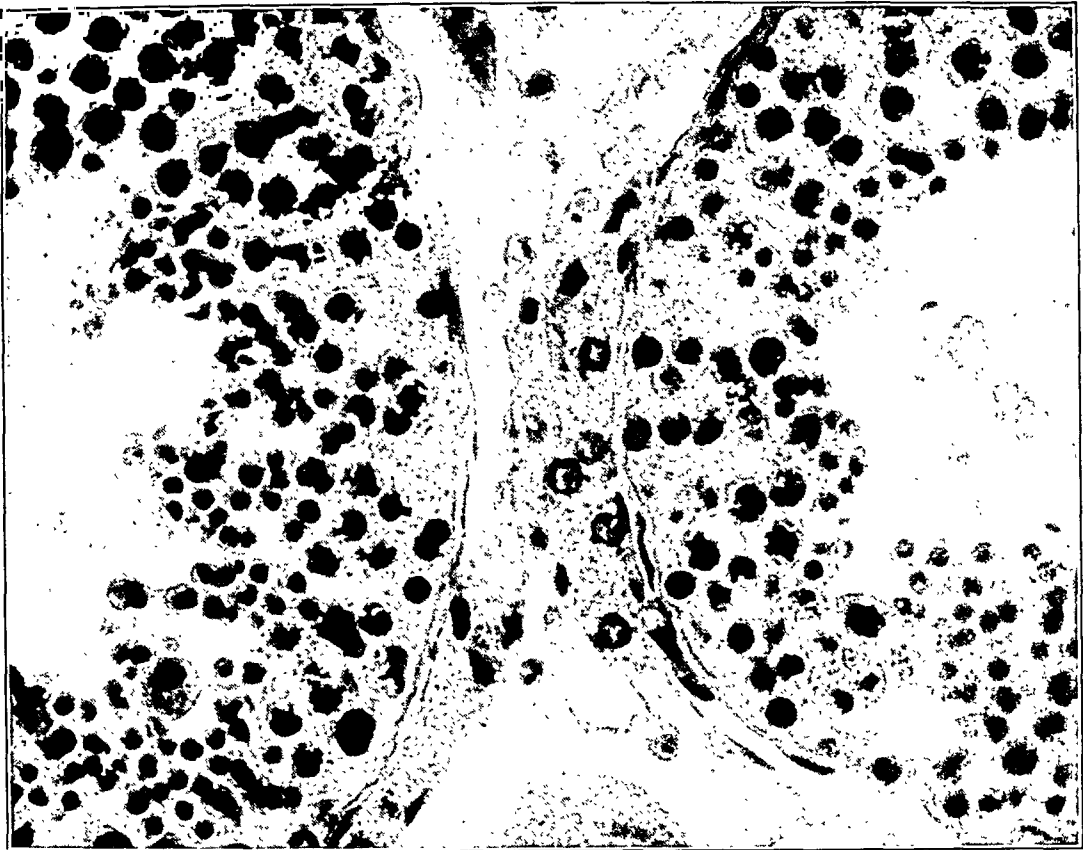
## DESCRIPTION OF PLATES

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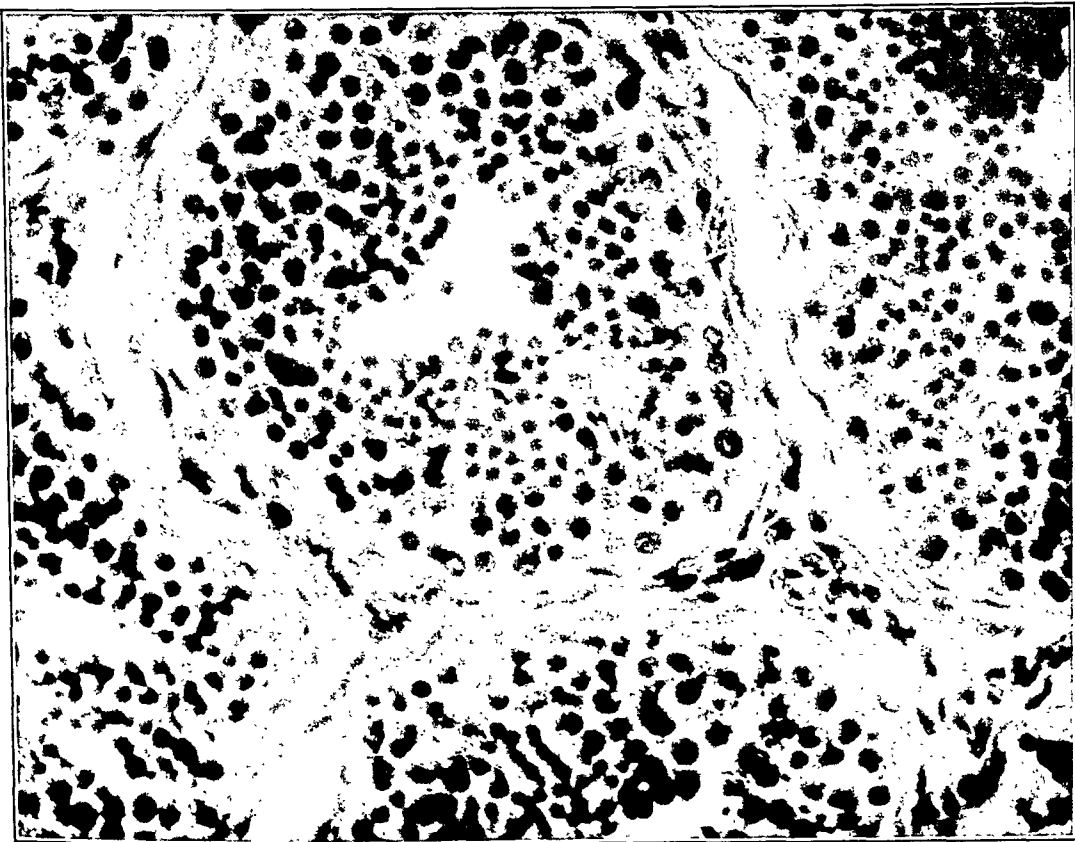
### PLATE I

FIG. 1. Case 1. The early effects of acute alcoholism upon spermatogenesis. Spermatocytes and spermatids increased in number, spermatozoa decreased in number.  $\times 465$

FIG. 2. Case 2. Marked increase in number of spermatocytes and spermatids with reduction in spermatozoa. Cell masses nearly occlude the lumina of the tubules.  $\times 325$



I

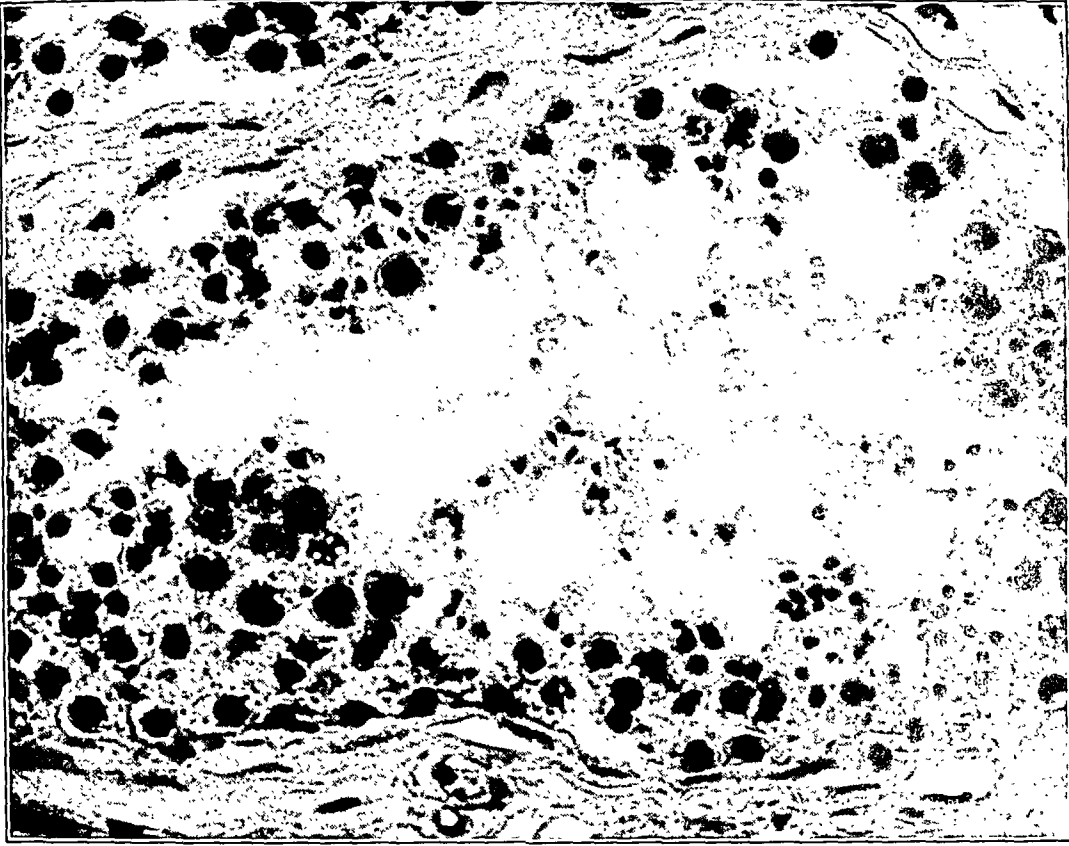


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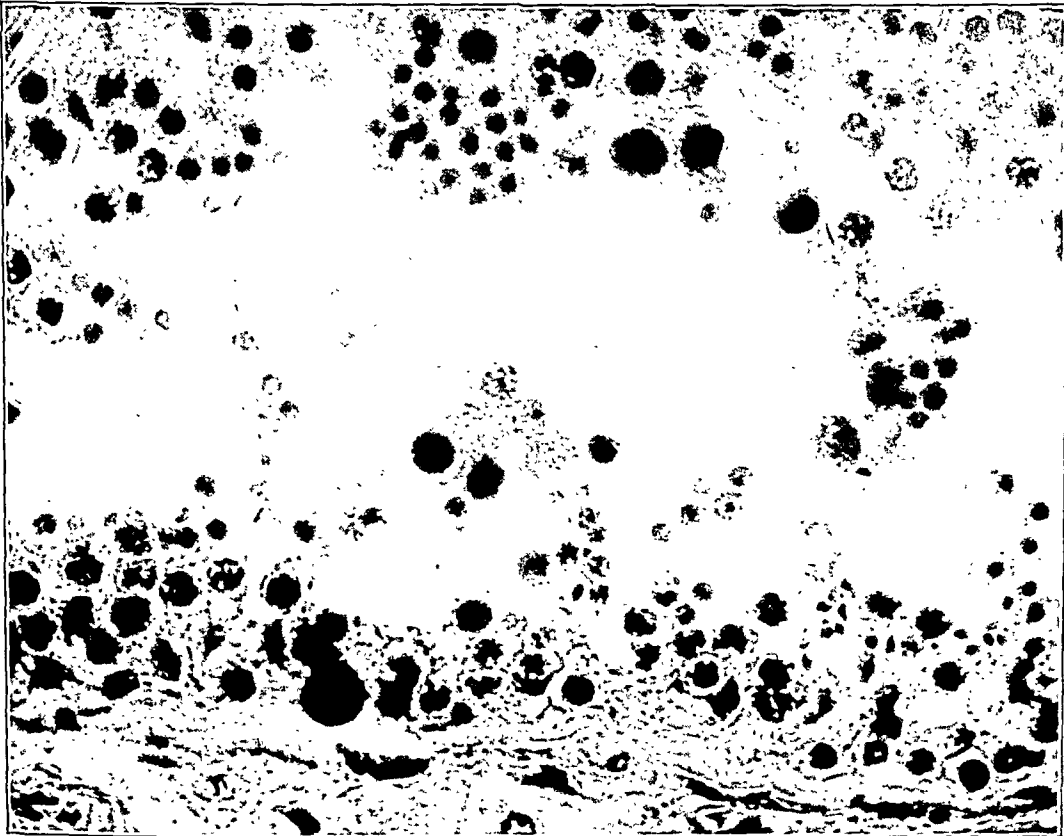
PLATE 2

FIG. 3. Case 3. Reduction of number of layers of epithelial cells by desquamation and vacuolation. Sperm heads with cellular débris in the widened lumen.  $\times 465$ .

FIG. 4. Case 4. Marked thinning of the epithelium with desquamation of spermatids and spermatocytes. Giant nuclei among the spermatocytes and also in the basal layer of the epithelium.  $\times 465$ .



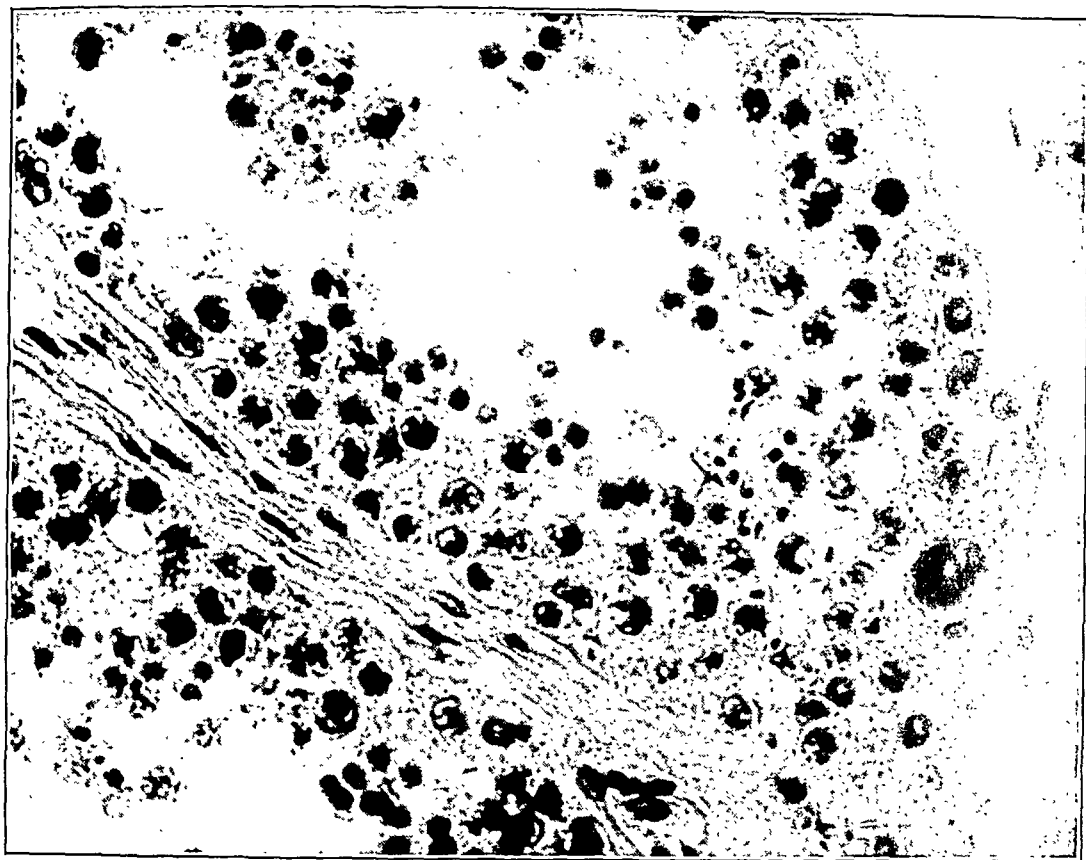
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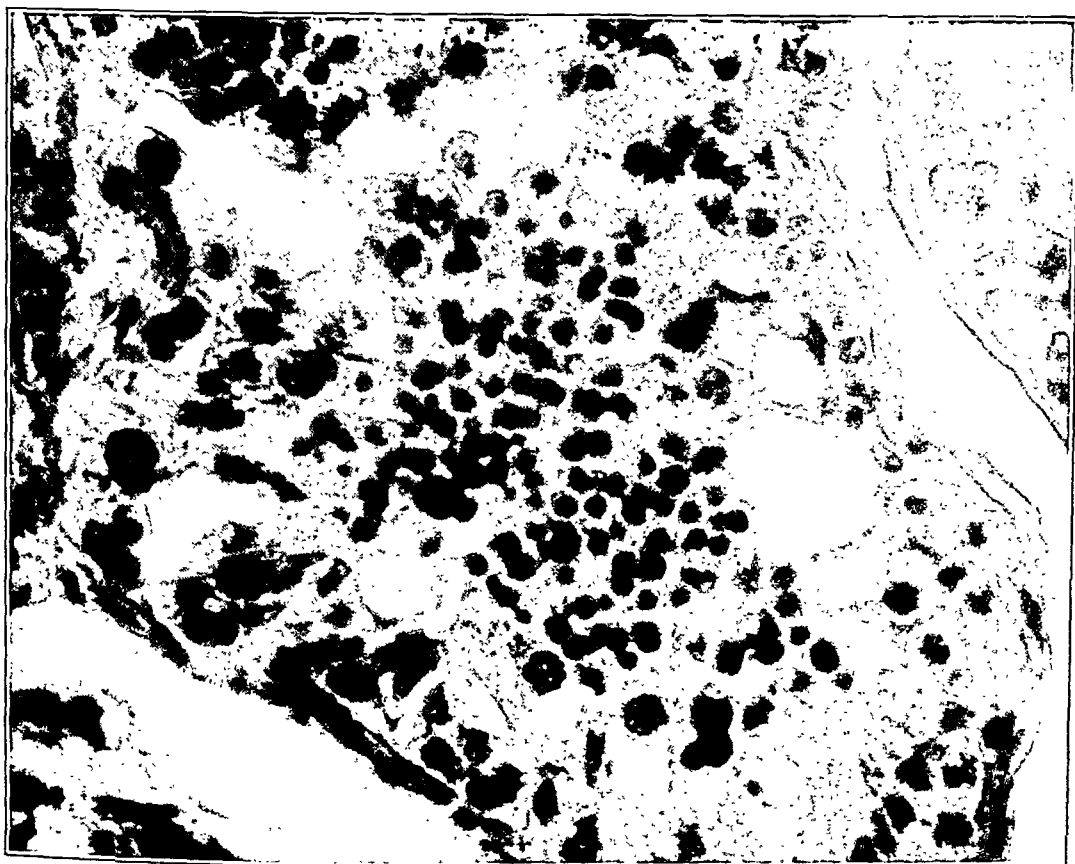
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PLATE 3

- FIG. 5. Case 4. Vacuolar degeneration of the epithelium with desquamation. Hyperchromatic giant nucleus in the basal layer.  $\times 465$ .
- FIG. 6. Case 5. Desquamation of spermatids and spermatocytes, filling the lumen with cells. Marked vacuolar degeneration of remainder of germinal epithelium.  $\times 465$ .



5



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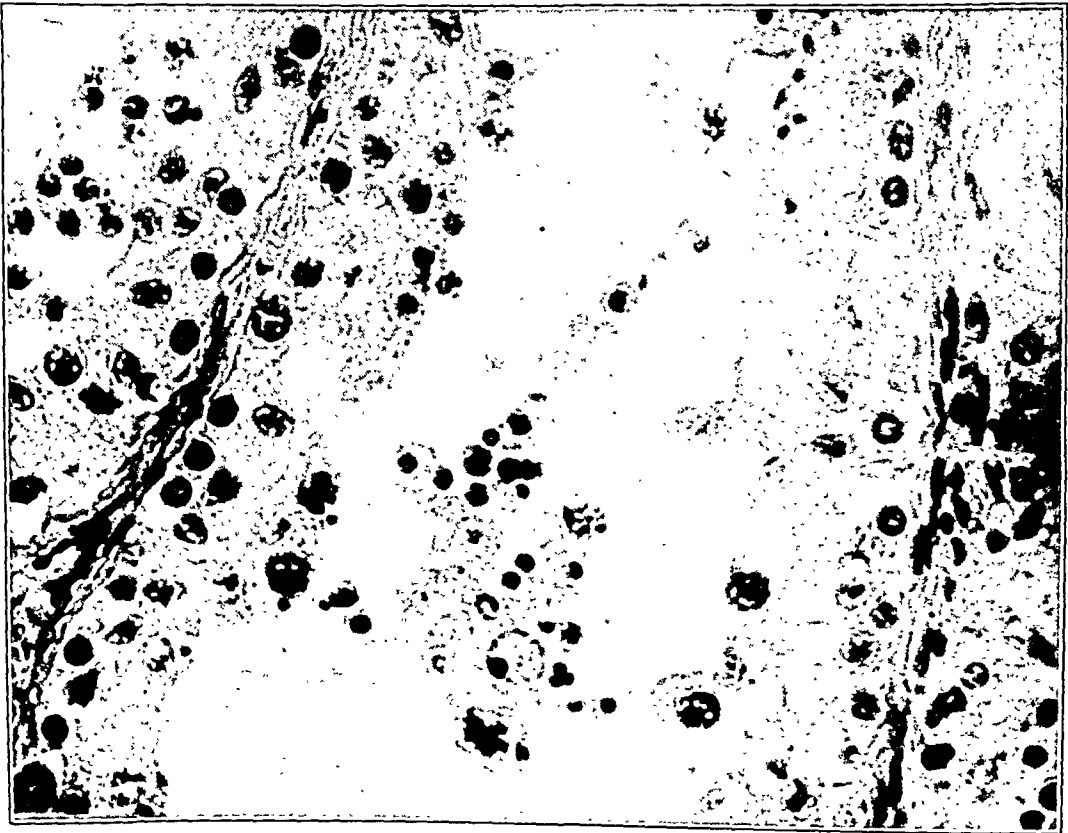
PLATE 4

FIG. 7. Case 6. Multinucleate teratocyte in tubule in lower center of field.  $\times 130$ .

FIG. 8. Case 6. Vacuolar degeneration and desquamation, these changes being more severe in this tubule than was general for this testis.  $\times 465$ .



7



8



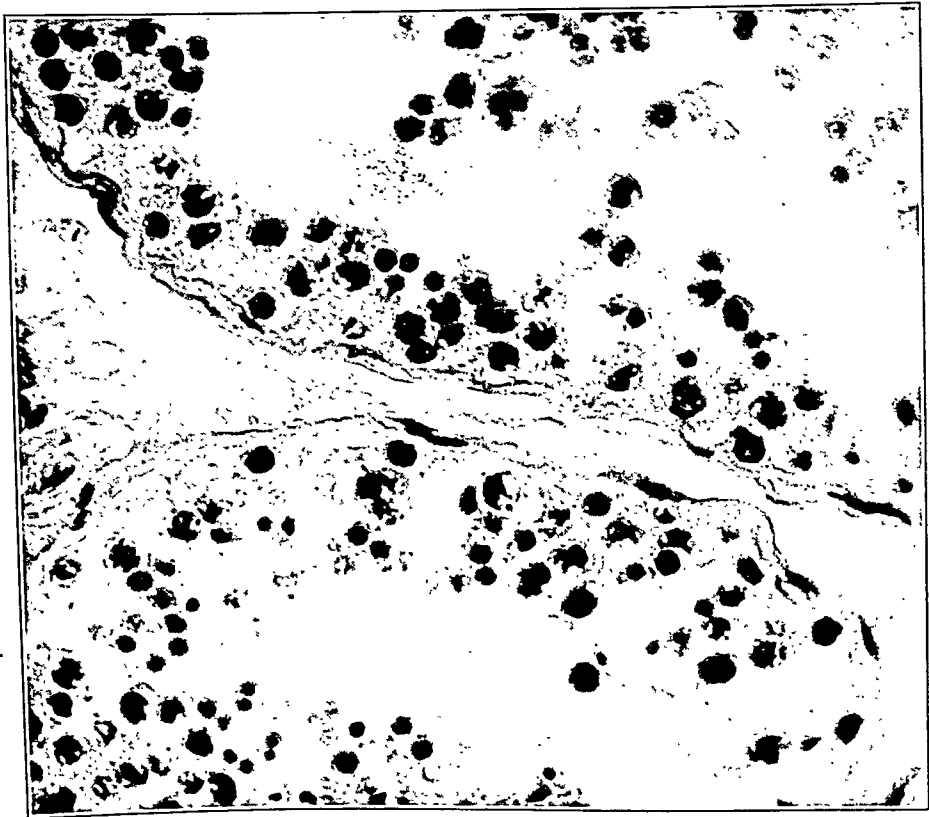
## PLATE 5

FIG. 9. Case 7. Hyperchromatic giant nuclei in the basal layer of the epithelium.  $\times 465$ .

FIG. 10. Case 8. Vacuolar degeneration and desquamation. Multinuclear teratocyte in upper portion of field.  $\times 465$ .



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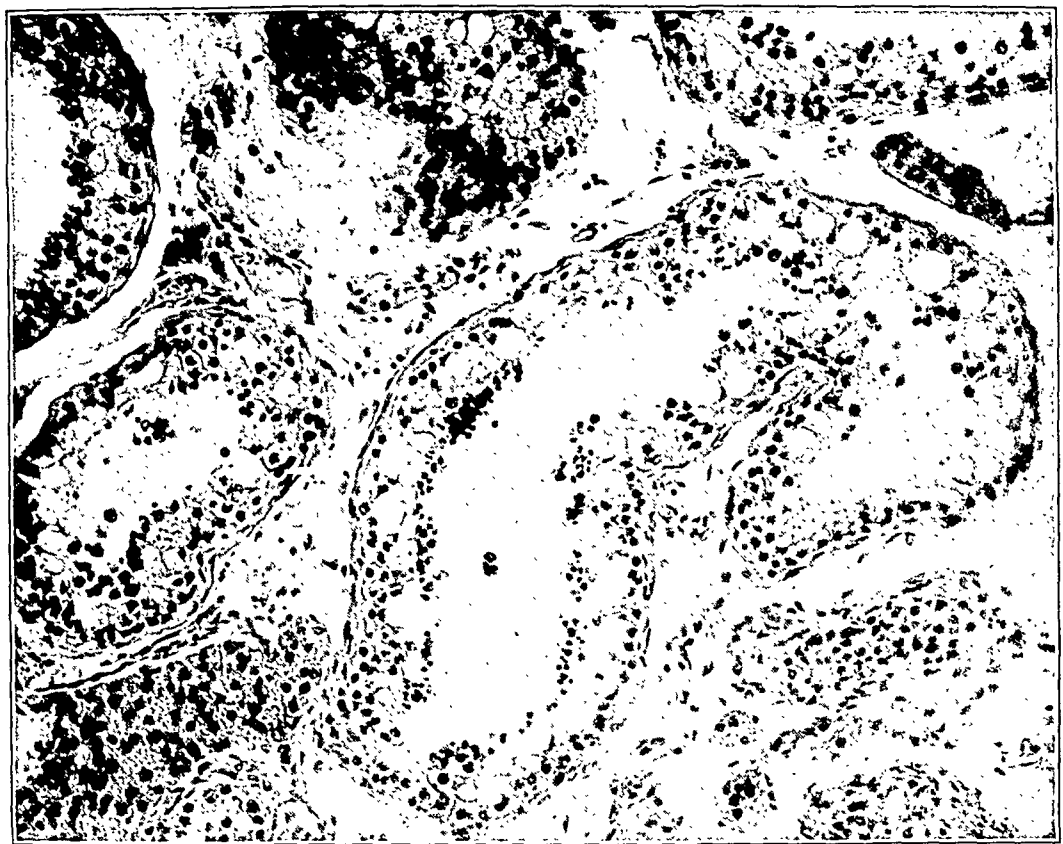


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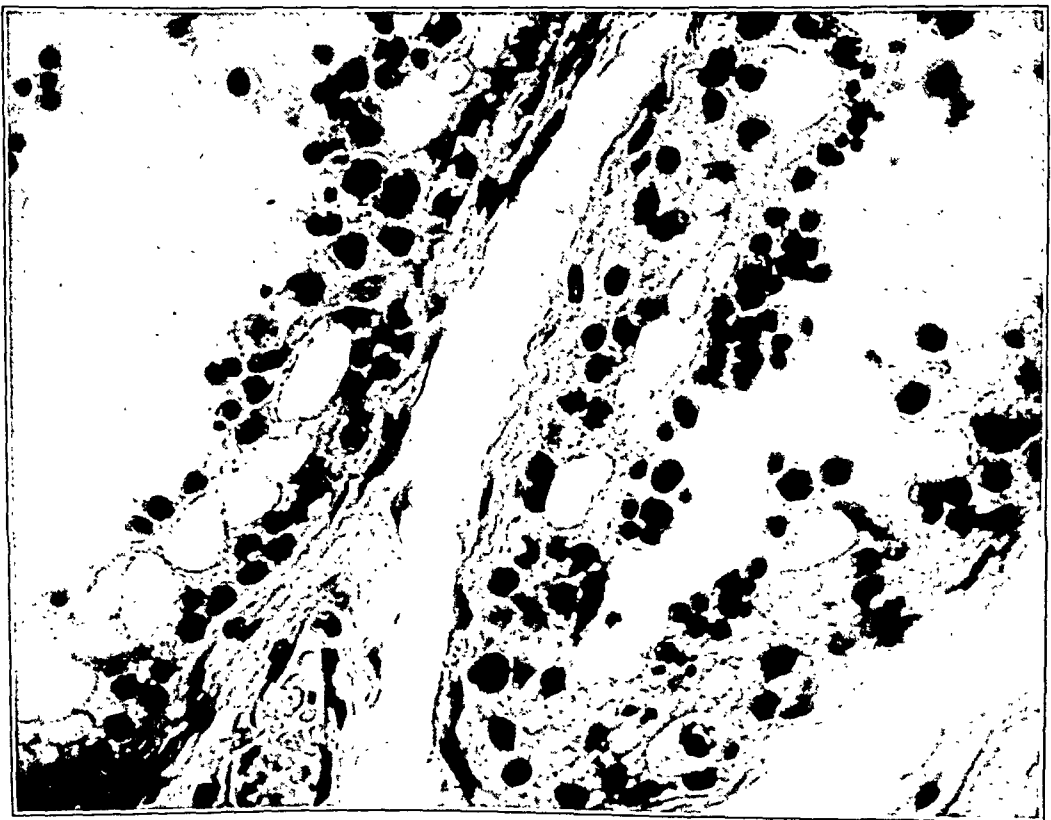
## PLATE 6

FIG. 11. Case 9. Lower power to show extreme degree of vacuolar degeneration of spermatocytes with marginally disposed non-vacuolated spermatids.  $\times 140$ .

FIG. 12. Case 9. Zonal vacuolar change with non-vacuolated spermatids and spermatocytes adhering to the wall. Numerous hyperchromatic nuclei, but not of "giant" size.  $\times 465$ .



11



12



## THE VENOUS DRAINAGE OF THE CAT SPLEEN \*

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As has been established in a previous communication,<sup>1</sup> the circulation through the spleen is an open one. The arterial system terminates in a widely distributed series of capillaries which in the dog, cat and sheep open out in a bell-shaped manner, and in the human in ampullary dilatations with oval-shaped apertures to discharge their contents into the pulp spaces. The pulp which represents the functional part of the spleen is formed by a vast network of reticulo-endothelial cells (pulp cells), supported by delicate threads of reticulin. The general construction is such that the elements of the blood are brought into intimate contact with the pulp cells, which have been shown by the author<sup>2</sup> to play an important part in the filtrative function exerted by this organ.

From the above findings it is obvious that the venous system is independent of the arterial system and manifests its chief function by draining the pulp spaces of their contents. Mall<sup>3</sup> has shown the emptying of the spleen is largely brought about by a contraction of the trabecular framework, which by virtue of its attachments to the walls of the veins pulls them open and at the same time compresses the pulp. During the contractions of the capsule and trabeculae a pressure may be built up in the venous system which is higher than that on the arterial side. Under these circumstances the blood no doubt is prevented from flowing back into the arteries by the concomitant compression of the ellipsoids with that of the pulp. The ellipsoids therefore function as check-valves for the arterial system. The rate of flow of blood through the normal dog spleen is not very great, being, according to Mall about 5 cc. per minute. I have found in perfusing cat and dog spleens with a pressure head of seventy-two inches of water on the arterial side and a back pressure of ten inches on the venous side, the spleen being fully distended, that the outflow of fluid from the vein averages 6 cc. per minute. This is fairly con-

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stant. On the other hand when the spleen is fully distended with the perfusing fluid and the back pressure on the venous side released, there is a sudden flow of the fluid from the vein out of all proportion to the rate of inflow. This keeps up until the pulp system is partially emptied. Following this the rate of outflow becomes normal, being as in the first case, the same as the arterial inflow. It is seen therefore that there is a considerable disproportion between the rate of inflow and the possible rate of outflow. Barcroft <sup>4</sup> has shown that considerable variations in size of the spleen may occur in the living animal. This he considers to be an emergency measure for the sudden flooding of the circulation with stored up blood elements from the spleen. These observations indicate a very free and intimate connection between pulp spaces and venous channels, much more so than between arterial system and pulp, else why the rapid emptying of the spleen as compared with its filling time? The communicating channels between the pulp spaces and the veins must therefore be numerous and of generous proportions. The study of this relationship of the venous system to the pulp in the cat spleen represents the subject matter of this paper.

The venous sinuses are readily recognized in sections of distended spleens, particularly in those of dogs and humans. In the latter their cubic content appears to be almost as great as that of the pulp itself. In his excellent phylogenetical study of the venous capillaries of the spleen Mollier <sup>5</sup> found that while there were certain fundamental principles of structure common to all, variations in the character of their walls in the different species were manifested. In the more primitive types the pulp tissue itself is used unchanged in this bounding process, the veins being nothing more than a continuous series of pulp spaces of uniform size. The higher types have more clearly defined walls made up of a specialized layer of endothelium. He has made an exhaustive study of these variations in structure and in all of them, even in the most highly developed, he has shown that their walls are incomplete. Fenestrations or stomata are present which communicate with the pulp spaces to allow for the ebb and flow of the blood elements between the venous capillaries and the pulp. The bulk of his discussion centered about the presence or absence of these openings in the capillary walls and the character of the lining cells which would allow for such fenestrations. Apart from the discussion on the structure of the walls of the veins, one would gather from his

paper that he believed the arterial and venous systems to be a unity, for he states that the venous capillaries "do not form an independent system but are merely canals (Gänge) formed in the pulp tissue." He believed the spleen to have a closed circulation except for the ebb and flow, as described above, through the sieve-like walls of the venous capillaries. This conception however in the light of our findings is untenable. The arterial capillary system is independent and is separated from the venous system by the pulp spaces. Having identified and described the terminations of the arterial system in the pulp I was anxious to demonstrate the beginnings of the venous system. As far as dog, human and sheep spleens were concerned I was unsuccessful. Recently however while examining sections of distended cat spleens with a Zeiss binocular microscope with stereoscopic attachments, I was able to identify definite expansions of many of the venous capillaries which I concluded were the beginnings of this system.

Fresh cat spleens were used. The collateral circulation was carefully tied off and cannulae inserted into the splenic artery and vein. The spleen was taken out and placed in warm normal physiological saline. To avoid the possibility of tearing the delicate structures of the pulp tissue it was found advisable first to perfuse the spleen by way of the artery with warm normal physiological saline. A pressure head of about seventy-two inches (water), on the arterial side was found necessary to establish the flow of the perfusing fluid. Finally before the spleen could be completely distended it was necessary to produce a certain amount of back pressure on the venous side. This was done by attaching a piece of rubber tubing to the cannula in the vein and raising the outlet ten inches above the spleen. When the spleen was fully distended and the return flow of fluid from the vein clear, the physiological saline supply was cut off and the cannula in the artery connected to a supply of Zenker's fluid at the same pressure head. The perfusion of Zenker's fluid was allowed to continue until all the tissues were thoroughly bathed in the fixative and the saline well replaced by the fixing fluid. The vein was then clamped off and the saline in the dish about the spleen replaced by Zenker's fluid. The spleen was then left with a slightly reduced pressure head of Zenker's fluid still maintained on the arterial side for some two or three hours. The artery was then clamped and the spleen left for twenty-four hours in the fixative. Blocks were carefully cut with a



sharp knife, washed, dehydrated and embedded in paraffin in the usual manner.

The spleen in its distended state is very spongy. It was found therefore advantageous to cut the sections at 20 to 25 microns. The thin filmy membranous walls of the veins in the cat spleen are very difficult to demonstrate unless properly stained. The most satisfactory stain, I found, was Heidenhain's iron hematoxylin, staining deeply and differentiating only sufficiently to take out some of the excess stain. No counterstain was found necessary or advisable. The minute structures of the venous system could then be demonstrated in a very striking manner in stereo with the Zeiss binocular microscope.

Tracing the venous system backward from the hilum, the large collecting veins before entering the spleen were found to be similar in structure to those in other parts of the body. Their wall consisted of an inner lining of endothelial cells supported by a thin muscle coat and outside of this a layer of loose areolar connective tissue. They were closely approximated by the branches of the splenic artery, artery and vein usually entering the spleen together. The general distribution of the arteries and veins throughout the spleen was, I found, as described by Mall.<sup>3</sup> That is, the veins and arteries tended to diverge and occupy positions within the splenic lobules quite separate from one another.

The entrance of the veins into the spleen usually occurred at a point where the trabeculae formed their attachment to the capsule, the capsule here being slightly invaginated. I was able to find however at certain points between trabecular attachments to the capsule that small branches of the hilum veins directly penetrated the capsule at an angle to open out into the adjacent pulp tissue. This however was not the usual finding. Of the three coats just described, only one, the inner lining layer of endothelial cells was found to continue on into the spleen. The muscle coat and adventitia stopped abruptly at the capsule. From this point on, the venous system through the spleen was found to be capillary in nature, consisting of a series of branching channels whose walls were made up of a single layer of endothelial cells. On entering the spleen the veins first pursued a course in the center of the trabeculae for variable distances. These represented the interlobular veins described by Mall. They were supported directly by the longitudinal muscle, elastic, and con-

nective tissue fibers of the trabeculae. A few short branches were given off to the surrounding pulp tissue. These were similar to the terminal branches and will be described later.

Tracing farther the interlobular veins in their course within the trabeculae one found that they very soon veered off into the pulp tissue proper. They are supported now only in part by the trabecular fibers. Looking at them in cross-section one finds that this support is anywhere from a small fraction to almost the whole of their circumference. Where supported, the endothelial cells are lying directly upon the trabecular fibers. Where unsupported, the walls consist merely of a single syncytium-like layer of endothelial cells in direct contact with the neighboring pulp cells. In the unsupported portions I was able to observe rounded or oval-shaped stomata which communicated directly with the neighboring pulp spaces. Their margins in many cases were everted, giving them an appearance very much like short, side branches which expand out in a bell-shaped manner to communicate with the pulp spaces. They were however not numerous and must play but a minor part in the drainage of the pulp as a whole.

Following the veins into the pulp I found that they soon became completely independent of the trabecular fibers as a supporting structure. Frequently however attachments between them were seen. After running a course of not more than 0.3 mm. the final branching occurred. These vessels being independent of the trabeculae and lying wholly within the lobule were called by Mall the intralobular veins. In their first part they consist of channels having thin walls made up of a single layer of syncytium-like endothelial cells supported directly by reticulum and the neighboring pulp cells. Their nuclei are oval and somewhat flattened. They are comparatively few in number. They appeared to have no definite arrangement except that their long axis was the same as that of the vessel. Their cytoplasm was drawn out into thin protoplasmic sheets, one cell blending with another in such a manner that it was impossible to distinguish them. Numerous rounded or oval-shaped stomata were found in their walls, very similar to those previously described.

Finally with the next system of branching, the end of the venous system was reached. These, functionally, are probably the most important branches. They vary from 0.1 to 0.5 mm. in length. Their walls in the first part are fairly well defined but as they reach the

terminus they end in such an indefinite manner that they are almost indistinguishable from the pulp cells. They are similar in structure to the branches just described but their stomata are larger and more numerous. As one traces a branch to its terminus one finds that the stomata become larger, gradually approximating those of the adjoining pulp spaces. In this way the stomata gradually become pulp spaces and the bounding cells, pulp cells. The terminus therefore may be very difficult to identify. Sometimes however the branches seem to end in a sort of an ampullary dilatation with numerous stomata opening out in all directions. Again one might find the walls gradually converging to terminate at a point. The stomata as before become larger as this point is reached. In all cases these branches were found to terminate in the pulp. I was unable to identify any direct communicating channels between artery and vein.

As we have been tracing the system backward the branches just described as terminal are, in truth, the beginnings of the venous system. These, I think, might be called the primordial branches to distinguish them from others which are more or less collecting channels. The obvious course of the blood flow from the pulp to the venous system is through the large stomata in the primordial branches. A certain amount, of course, must also flow directly from the pulp through the stomata of the intralobular veins and interlobular veins where not ensheathed by the trabeculae.

Mollier's explanation of the circulation through the spleen as being through a continuous system of tubules is therefore wrong. The venous system is independent as is also the arterial system. His description and study of the phylogenetical development of the venous capillaries is very complete and I quite agree with his conception of the structure of their walls as seen in the various animals. While he mentions the fact that he has made a study of cat spleens as well as those of many other animals, he neglects in his text to comment particularly on their structure. It may have been that the smallness of the venous system in the cat made it unattractive for detailed study. This however has been a feature which has enabled me to find the solution of the beginnings of this system.

Because the veins in the cat have been shown to have definite beginnings in the pulp spaces does not prove that such a condition exists in other mammals. In the human, sheep, ox, rabbit, guinea pig and dog spleens one finds a very extensive system of venous cap-

illaries which, particularly in the human, almost overshadow that of the pulp. In some animals, as Mollier has shown, the veins consist merely of "retiform spaces following upon one another and reduced to the same calibre to form connected net-like tubes with perforated walls." In others, particularly in the human spleen, they have well defined walls made up of a protoplasmic syncytial lattice of longitudinal bands united by transverse bridges and supported by a reticulum; a perforated structure having stomata of various sizes and shapes. The stomata however are so numerous and of such size that one can readily conceive of an adequate flow of blood elements through them from the pulp to the venous system without the necessity for any definite beginning to the system. On the other hand, in the cat the venous capillaries are short and for the most part bounded by a protoplasmic lining with very few stomata. Such a system must have an adequate number and size of openings to allow for a sufficient flow of blood from the pulp to veins when the necessity arises. These I think I have demonstrated quite conclusively in the large stomata of the primordial branches which have their beginnings *de novo* in the pulp.

### SUMMARY

1. The venous capillary system in the spleen of the cat has been shown to have definite points of origin in the pulp.
2. The venous capillary system in the spleen of the cat is independent of the arterial system.
3. The independence of the venous capillary system as seen in the cat spleen may be taken as further proof of the open circulation of the spleen.

NOTE: I am indebted to Professor Klotz for much helpful advice and guidance in the conduct of this work.

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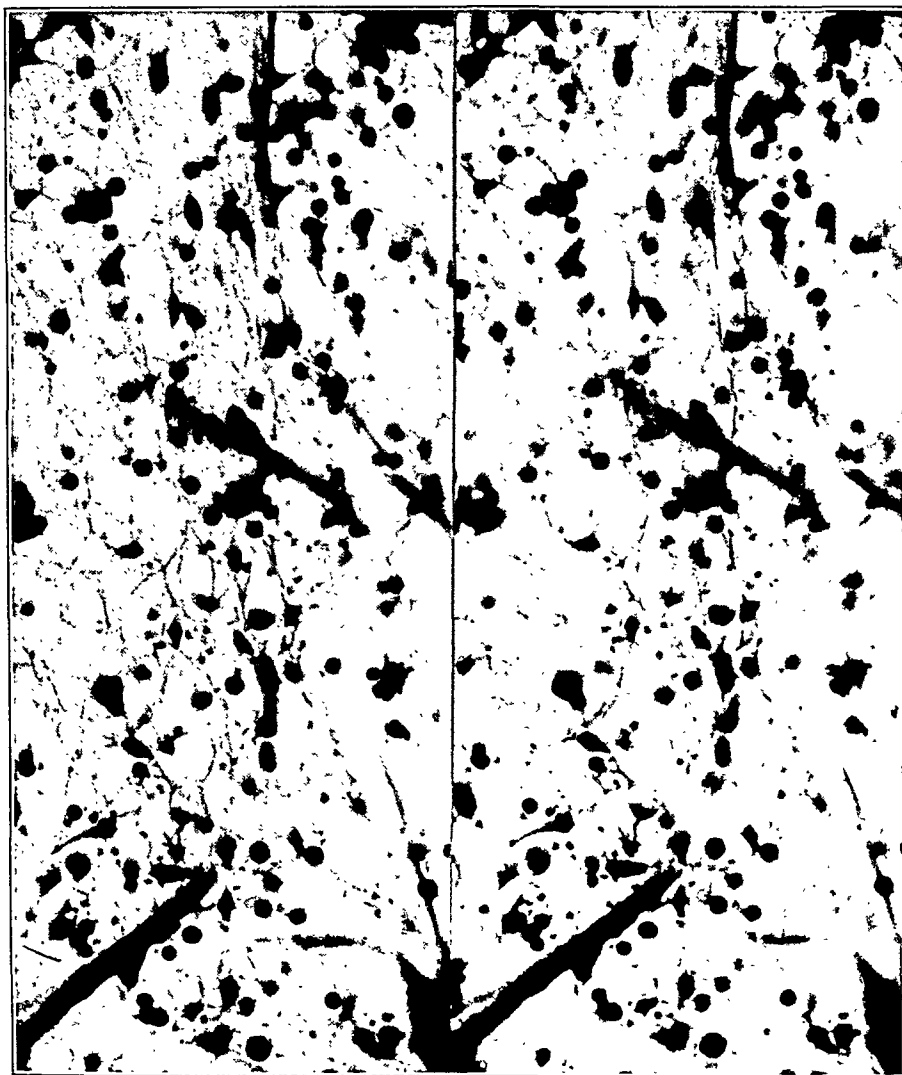
## DESCRIPTION OF PLATES

NOTE: The illustrations may be viewed stereoscopically by placing the narrow edge of a blotter midway between the two pictures, the nose touching the opposite edge. Two pictures are seen at first but if one continues to focus the eyes on them they will blend into one picture giving a three-dimension view.

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### PLATE 7

FIG. 1. A primordial vein of the cat's spleen, illustrating its indefinite point of origin in the pulp. Note the large stomata in its first part.  $\times 400$ .

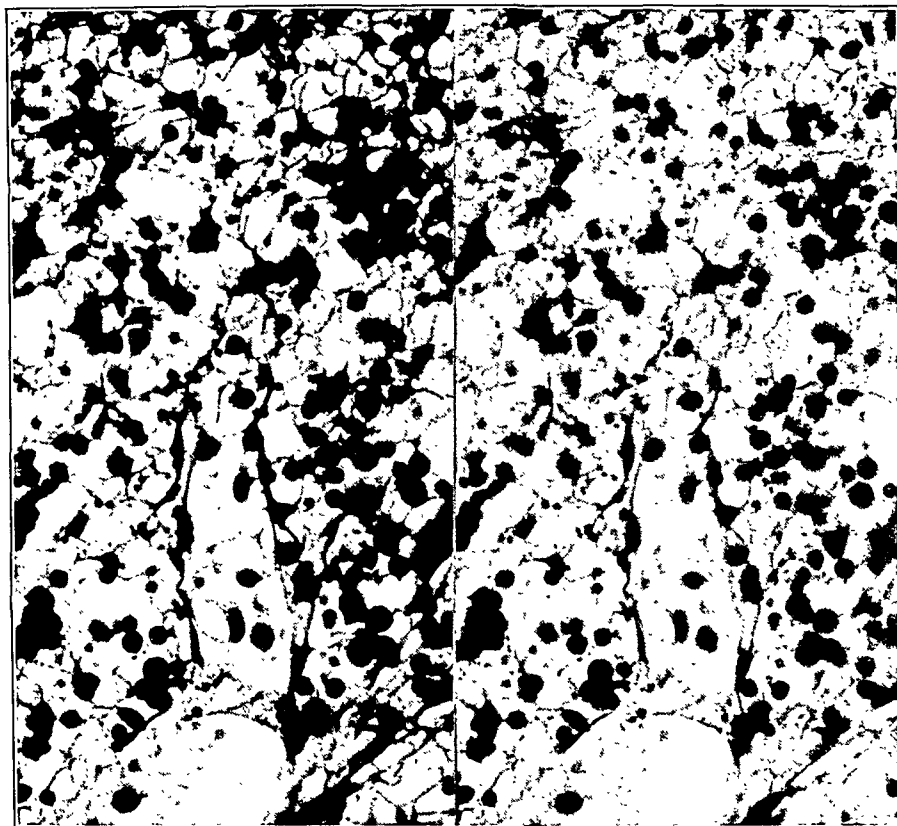


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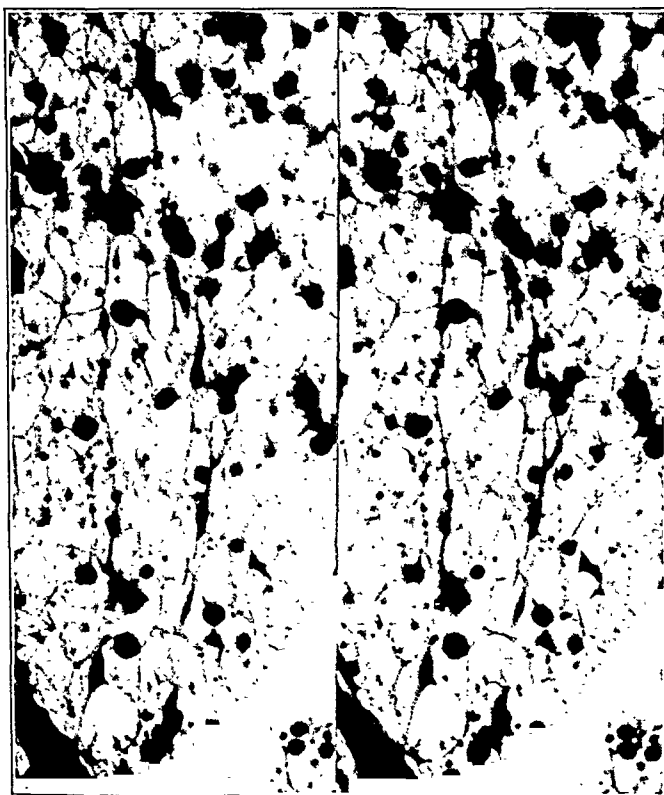
PLATE 8

FIG. 2. A short primordial branch beginning in an ampulla-like dilatation and showing numerous large stomata in its wall.  $\times 400$ .

FIG. 3. A primordial branch having a more or less definite point of origin in the pulp. From here the walls gradually expand to the full diameter of the vein. Numerous large stomata are seen in its wall.  $\times 400$ .



2



3





## NEURO-EPITHELIOMA (GLIOMA) OF RETINA WITH METASTASES \*

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Cases of neuro-epithelioma (glioma) of the retina with metastases to organs are rather rare. In the following two cases a striking picture of metastases was found at the postmortem examination. The findings in the first case are especially valuable, since it was possible to carry on the autopsy to its utmost completeness.

**CASE 1. *Clinical History:*** A Chinese boy, 4 years old, came to Peking Union Medical College Hospital April 30, 1925, because of a protruding mass in the left eye. His family history was negative. Two or three years earlier he had had smallpox. Following the attack of smallpox a scar was seen in the cornea of the left eye which was at that time found to be "blind." Three and a half months before admission a tumor appeared in the left eye, accompanied by local discomfort. This tumor grew rapidly, reaching the size of a man's fist at the time of admission. Physical examination showed the child to be underdeveloped and undernourished, with the left eye completely destroyed by a large protruding tumor mass (Fig. 1). The right eye was normal. On the head were three small masses; one in the left frontal region, another in the left temporal region, and the third in the left preauricular region. Cervical lymph nodes were moderately enlarged. On May 1, 1925 the tumor of the left eye was removed by exenteration of the orbit.

### PATHOLOGICAL REPORT

Specimen is that of a tumor of the left eye, soft in consistency, red or dark red in color and measuring 8 by 6.5 by 6 cm. The eyeball is not recognizable. A portion of the skin of the upper eyelid is attached to the tumor. The surface of the extra-orbital portion of the tumor is rough and covered with fibrinopurulent exudate. On section the cut surface is generally soft and dark red with a few scattered areas of yellow, but in the center there is one grayish white area measuring about 2.5 by 1 cm.

***Microscopic Examination:*** Microscopically, under low magnification, the tumor appears very cellular with only a small amount of fibrous stroma. In the tumor tissue there are many areas of necrosis

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and hemorrhage. The necrosis involves especially the cells which are a short distance away from the blood vessels. Under high magnification, two types of tumor cells are seen, both showing numerous mitotic figures. The first type (Fig. 15), to which the majority of the cells of the tumor belong, are small, rounded and lymphocyte-like with only a small amount of cytoplasm, and with nuclei which are round and deep-staining. The other type (Figs. 10, 11, 12, 13 and 14) consists of elongated or columnar epithelial-like cells arranged as a rule in single rows. Their nuclei are oval or elongated and at the poles of each there is found a moderate amount of cytoplasm. The cytoplasm at the base of the cell sometimes stretches out to form a pseudopod of varying thickness, which is attached to a thin band of fibrous tissue or to the wall of a blood vessel. The cytoplasm at the opposite end of the cell shows only slight irregularity of the free surface caused by the presence of small protoplasmic excrescences. The free surface of these cells is frequently covered with either round or necrotic cells, or is in close approximation with the free surface of the similar epithelial-like cells which are arranged in the opposite direction. These cells frequently tend to form curved rows or rosettes. Those which form the rosettes usually have no pseudopods nor fibrous nor vascular attachments. Each rosette consists of from ten to thirty or more cells. The lumina of many of the rosettes contain loose cells, both living and necrotic. No typical rods and cones are found. Neuroglia fibers are not demonstrated by the phosphotungstic acid hematoxylin stain.

*Diagnosis:* Neuro-epithelioma (glioma) of retina with extrabulbar extension.

On May 3, 1925, a fluctuating swelling, 3.4 by 2.2 by 0.5 cm., appeared in the right superciliary region. Following this, numerous other swellings appeared. A photograph of the patient on June 6, 1925, is shown in Figure 2. On June 25, 1925, the patient died.

#### AUTOPSY REPORT

The body is that of a well developed but greatly emaciated Chinese boy weighing 7285 gm. and measuring 88 cm. in length. The left eye is absent with the optic cavity partly filled with soft, gray, necrotic tissue. The right eye is pushed downward by a tumor mass 6 cm. in diameter which extends up into the right superciliary region. The cornea shows a small ulcer in the center. The lower lid is

swollen. Over the left frontal eminence is a tumor mass 5 cm. in diameter. Another mass the size of a walnut is in the left preauricular region. A few others are palpable underneath the scalp along the parietal and the parietofrontal sutures. Only the cervical and the inguinal lymph nodes are palpable, some of these being about 2 cm. in diameter. The left parotid gland is large and infiltrated by tumor tissue.

*Peritoneal Cavity:* The peritoneal cavity is normal except that the retroperitoneal lymph nodes are greatly enlarged, some of them along the pelvic brim reaching 4 cm. in diameter (Fig. 9). They are filled with white soft tumor tissue which on section shows areas of necrosis and hemorrhage.

*Pleural Cavities:* On each side of the spinal column and on the inner surfaces of the ribs are several white or hemorrhagic, soft tumor masses either rounded or irregular, each well covered by the parietal pleura. The largest mass is 5 cm. in diameter.

*Skull and Dura:* On separating the scalp from the calvarium large, soft, hemorrhagic tumor masses are exposed. The largest measuring 5 cm. in diameter is in the frontal bone and the others which are smaller are found along the cranial sutures (Figs. 3 and 4). These masses have worn through the calvarium and are deposited on the external surface of the dura (Fig. 5). The inner surface of the dura shows only two rounded tumor masses, each measuring about 2 cm. in diameter, and projecting into the left frontal lobe of the brain. When the dura was removed these two masses were left buried in the brain. The superior longitudinal, the left lateral, and the left superior and inferior petrosal sinuses are filled with thrombi.

*Brain:* (Fig. 6). Large and small areas of hemorrhage are found on the surfaces of the left frontal lobe and the anterior portion of the right parietal lobe. The superficial cerebral veins in these hemorrhagic areas are mostly filled with firm, grayish white, inelastic thrombi. The two tumor masses which broke away from the dura when the latter was removed are found in the left frontal lobe, one situated in the posterior portion of the superior frontal gyrus, and the other in the anterior portion of the middle frontal gyrus. The brain tissue in the immediate vicinity of these tumor masses is greatly compressed and shows multiple hemorrhages. Sections made through the entire brain show wide-scattered hemorrhages in the fissures and in the adjoining brain substance. The hemorrhage is especially marked

in the left temporal lobe, in which the brain substance has been converted into a soft, dark red mass. The right optic nerve is normal in gross. The entire left optic nerve, except for 1 mm. near the optic chiasm, is infiltrated with tumor and the extracranial portion of the nerve is necrotic.

*Spinal Cord:* The spinal cord is normal. In the upper sacral region a few small masses of soft white tumor tissue are present outside of the dura and in the pia-arachnoid.

*Bones:* The tumor masses in the calvarium have already been described. In the head the tumor tissue is also found in the body of the sphenoid bone, the sinus of the frontal bone, the basilar process of the occipital bone, the bones of the left orbit and the mandible. In the latter the tumor masses are found as subperiosteal swellings on the external surface of the left ramus and on the external and internal surfaces of the right ramus. Longitudinal sections of all the bodies of the vertebrae show on the cut surface large and small areas of tumor tissue, irregular, opaque and yellowish white, in marked contrast to the red color of the surrounding myeloid tissue (Fig. 9). Similar opaque, yellowish white or gray areas are also found in the following bones: humeri (Fig. 8), scapulae, ribs, sternum, pelvic bones, femurs (Fig. 7) and calcanei. No evidence of tumor is found in the other bones. The tumor in the scapulae and the ilia has lifted up and worn through the periosteum, and extended into the adjoining muscles (the supraspinatus and the infraspinatus, the subscapular, the gluteus medius, and the iliopsoas). In one humerus, and in both femurs the tumor has also worn through the cortex, forming subperiosteal swellings. All other organs are normal.

*Microscopic Examination:* The metastatic tumors in different localities (Figs. 16, 17, 18 and 19) show histological characteristics essentially similar to those of the primary tumor except that no rosettes are found, although there is a general tendency for the tumor cells to become elongated, especially in the bone marrow. The perivascular arrangement of the tumor cells, the necrosis and the hemorrhage are very conspicuous in some places. The amount of stroma is small, and in many places is represented by only a few delicate fibers radiating from the wall of the blood vessels of the tumor.

*Anatomical Diagnoses:* Neuro-epithelioma (glioma) of retina with metastases to eyelids, left optic nerve, dura, meninges of spinal cord;

bones (frontal, parietal, occipital, sphenoid, bones of the orbit, mandible, scapulae, ribs, sternum, humeri, femurs, calcanei, bodies of vertebrae); muscles (supraspinatus, infraspinatus, subscapulae, gluteus medius, iliopsoas); lymph nodes (cervical, retroperitoneal, inguinal); pleura, and parotid gland; and thrombosis of cerebral veins with extensive hemorrhage in the brain.

**CASE 2. *Clinical History:*** A German boy, 3 years old, was admitted to Peking Union Medical College Hospital on Nov. 11, 1921 because of protrusion of the left eyeball for eighteen months. The family and past histories were unimportant. Eighteen months earlier his parents noticed a very faint yellowish spot in the pupil of his left eye which was then found to be blind. Six or seven months before admission this eye became more protruding. Frequent vomiting and nausea started three weeks ago. Physical examination showed the left eyeball to be conical in shape and protruding (Fig. 10), the anterior chamber shallow, the pupil round, widely dilated, no reaction to light, lens opaque and dislocated upward and to the temporal side. Operation for the exenteration of the orbit was done on Nov. 12, 1921. Toward the end of the operation patient stopped breathing and died.

### PATHOLOGICAL REPORT

Eye slightly enlarged. Horizontal diameter 24.5 mm., anterior-posterior diameter 27 mm. Cornea clear and conical. Pupil dilated, immobile and slightly eccentric. Anterior chamber shallow. Posterior sclera invaded by pinkish gray tumor tissue. On equatorial section a tumor is found filling up the vitreous space and firmly attached to the retina and choroid on one side near the equator. Posteriorly the tumor covers the retina and the optic disc; anteriorly it covers the lens and the ciliary body. Behind the retina there is a layer of tumor tissue 1.5 mm. thick. The optic nerve is thick, friable and infiltrated by tumor tissue which is also present in the tissue removed from the optic cavity.

***Microscopic Examination:*** Microscopically the tumor is found to be very cellular, consisting of closely packed cells with rounded or oval nuclei and a very small amount of cytoplasm. There are many mitotic figures (Fig. 22). No rosettes are found. The cells are well preserved around the blood vessels which are present in large numbers, but those not in the immediate vicinity of the vessels are necrotic (Fig. 21). The retina is entirely destroyed by the tumor in the region about the optic disc, but farther away it is still intact. The vitreous is largely filled with tumor. The choroid is destroyed in places and its branching pigmented cells may be found scattered in

the thick layer of tumor tissue between the pigmented epithelium and the sclera. The sclera is intact, although thinned out in places. No point is found at which the tumor definitely penetrates the sclera. The tumor is present, however, outside of the sclera, enclosing the whole posterior aspect of the eyeball. The optic disc and the optic nerve are infiltrated by tumor and their normal structures are no longer recognizable. The tissue removed from the optic cavity also shows tumor.

*Diagnosis:* Glioma of retina of the left eye with extension into the left orbit.

### AUTOPSY REPORT

The body is that of an emaciated white boy, weighing 9 Kg., and measuring 84 cm. in length. The left orbit is exenterated. The right eye is normal. None of the organs is remarkable except for the following condition noted in the central nervous system: The surface of the cerebral cortex is covered with many minute grayish islands of tumor tissue in the pia-arachnoid, more marked along the vessels in the sulci than over the surface of the convolutions. At the base of the brain and on the surface of the cerebellum, pons and medulla, these islands coalesce and form larger and thicker patches varying from a few millimeters to 3 or 4 cm. in diameter (Fig. 23). Anterior to the optic chiasm is a soft, gray, oval tumor mass about 1.6 cm. in length, and 1 cm. in width and in thickness, pressing upon both the left and right optic nerves and on the chiasm itself. On section the superficial portions of the cortex of cerebrum and cerebellum are found to be invaded by small nodules of tumor from the meninges. The lateral ventricles are slightly dilated. The ependymal surface is covered by numerous rounded or flattened, single or conglomerate tumor nodules measuring up to 5 mm. in diameter. Larger tumor masses are found in the choroid plexus. The intra-orbital portion of the right optic nerve is infiltrated with tumor. The spinal cord, especially its lower half, is greatly thickened and fills the dural cavity tightly. Its pia-arachnoid is extensively infiltrated by soft, grayish tumor tissue. On section the cut surface shows the tumor in the meninges invading the cord from all sides, especially from the dorsal side. A few small tumor masses are also found in the cauda equina.

*Microscopic Examination:* The tumors in the brain and spinal cord (Figs. 24, 25, 26, 27 and 28) show the same type of cells as those

of the primary tumor in the left eye. Most of the secondary growth is found in the pia-arachnoid, covering the larger part of the surface of the brain and the cord. In some places it extends directly into the brain or the cord from the surface. In others it fills up the Virchow-Robin's spaces and travels with the blood vessels into the deeper nervous tissue. The choroid plexus is almost entirely replaced by the tumor. The spinal nerve roots are either completely surrounded by or heavily infiltrated with tumor. There is no evidence of tumor in the right eye, but the right optic nerve is found to be completely surrounded by a layer of tumor tissue.

*Anatomical Diagnoses:* Glioma of retina of left eye with extension to the left orbit, brain, choroid plexus, spinal cord, meninges and the right optic nerve.

### DISCUSSION

Tumors of the retina, as illustrated by the above two cases, have long been called gliomas. It is, however, generally recognized that the glioma of the retina is quite different from the ordinary glioma of the brain in its rapid growth, greater tendency toward metastasis and its lack of glia fibers demonstrable by ordinary staining methods. In 1891 Flexner<sup>1</sup> first showed the external granular layer of the retina as the origin of the tumor and called attention to the fact that cells of the "rosettes" corresponded to the rod cells and cone cells of the retina. He therefore proposed the name neuro-epithelioma of retina, which was adopted by Wintersteiner<sup>2</sup> in 1897. Lately, however, the term retinocytoma has been suggested by Mawas<sup>3</sup> on account of the close resemblance of the neoplastic cells to the undifferentiated cells of the embryological retina. On the other hand, Urta<sup>4</sup> and Ascunce,<sup>5</sup> having succeeded in demonstrating the presence of glia fibers in this type of tumor, are in favor of calling it by its previous name — glioma. Finally, Bailey and Cushing,<sup>6</sup> who are inclined to believe that the cells of the rosettes are primitive spongioblasts, have named the tumor containing rosettes spongioblastoma primitivum retinae and that containing neuroglia cells or nerve cells (both of which are derived from the retinoblasts) retinoblastoma.

This wide variation in terminology is largely due to the fact that this type of tumor is essentially an embryonal multipotent new-growth. While its cells generally are not sufficiently differentiated to give one any definite information as to its true nature, it may in



various cases produce neuroglia cells, rod cells and cone cells, or even nerve cells.<sup>7</sup> This fact at once forces one to regard the retinal epithelium as the origin of the tumor, for it is only from this structure that all these elements can develop. The embryonal character of this type of tumor is further illustrated by the morphological resemblance of the tumor cells to the cells of the embryonal retina, as already pointed out by Collins<sup>8</sup> and Mawas, by its rapid growth, and by its occurrence chiefly in young children.

If this type of tumor is recognized as originating from the embryonal retina, it then becomes a simple matter to account for its microscopic variations — the presence of the undifferentiated cells, the difficulty with which the glia fibers are demonstrated by ordinary methods, and the inconstant presence of the neuroglia, neuroepithelial or nerve cells. The presence of rosettes in the primary tumor of Case 1 and their absence in the metastases can then be explained by the more complete differentiation of the cells of the primary tumor than of those of the metastatic growths.

It also becomes apparent that although we are still employing such terms as neuro-epithelioma or glioma of the retina, they are inadequate to designate this type of tumor, for such terms express the unipotent nature of the tumor which is, as a matter of fact, multipotent.

Since the retinal epithelium is formed by the budding off of the medullary epithelium which later develops into the central nervous system, we may expect to find tumors of the brain and the spinal cord which are analogous to those found in the retina. This is indeed the case. In the so-called neuro-epithelioma of the brain, structures similar to the rosettes of the retinal tumors are found. In the tumors called medulloblastoma by Bailey and Cushing both neuroglia cells and neuroblasts may be identified. These tumors, furthermore, present the same embryonal characteristics of the retinal tumor in their rapid growth, lack of cell differentiation, inconstant presence of one or another element and tendency toward spreading into the meninges.

In regard to metastasis Wintersteiner gives the frequency with which the various organs are involved as follows:

TABLE I

Brain and meninges. . . . .	43 times
Skull and bones of face. . . . .	40 "
Neighboring lymph nodes. . . . .	36 "
Parotid. . . . .	9 "
Skeletal bones. . . . .	9 "
Liver. . . . .	7 "
Spinal cord and meninges. . . . .	5 "
Kidneys. . . . .	2 "
Ovaries. . . . .	2 "
Lungs. . . . .	1 time
Spleen. . . . .	1 "

To the above table the following cases of metastases to the distant organs may be added from the literature:

TABLE II

Brain and meninges. . . . .	2	(Knapp, <sup>9</sup> Taylor and Fleming <sup>10</sup> )
Skull and bones of face. . . . .	3	(Keys, <sup>11</sup> Knapp, <sup>9</sup> Taylor and Fleming <sup>10</sup> )
Distant lymph nodes. . . . .	2	(Fehr, <sup>12</sup> Taylor and Fleming <sup>10</sup> )
Skeletal bones. . . . .	4	(Fehr, <sup>12</sup> Gardiner, <sup>13</sup> Knapp, <sup>9</sup> Taylor and Fleming <sup>10</sup> )
Liver. . . . .	2	(Knapp, <sup>9</sup> Radcliffe and Goldberg <sup>14</sup> )
Lungs. . . . .	1	(Fieber <sup>15</sup> )
Testes. . . . .	1	(Gardiner <sup>13</sup> )

From the above tables it will be seen that metastases in distant organs by way of the blood stream and the lymphatic stream are not common, as there are only twelve cases in which the skeletal bones are involved as in Case 1. On the other hand, secondary growths in the head are much more frequent and this is generally attributed to the direct extension of the tumor. This is certainly true in Case 2 and probably so to a certain extent in Case 1 in which the head is especially involved. In Case 2 the spreading of the tumor by direct extension is as definite as it is remarkable. Starting from the eyeball, the tumor at once becomes widespread without breaking the continuity in the entire central nervous system. Its distribution reminds one of that of the cellular exudate in cases of acute meningitis, or that of the fluid material artificially injected into the meningeal spaces. This mode of spreading is possible, however, only when the tumor is rapidly growing and when the stroma is insufficient to keep the loose tumor cells from spreading readily into places offering the least resistance.

Attention also may be called to the fact that from the above tables there are only five cases recorded in which the spinal cord and its meninges are involved as in Cases 1 and 2. In view of the frequent involvement of the brain, the small number of cases of cord involvement reported is probably due to the fact that the cord is not always examined at the time of autopsy.

### SUMMARY

Two cases of tumor of the eye, commonly known as glioma or neuro-epithelioma of the retina, are reported: one with metastases to the skull, skeletal bones, muscles, lymph nodes and meninges; and the other with extension to brain, spinal cord and meninges. The embryonal character of these tumors and their analogy to certain tumors of the central nervous system are stressed.

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## DESCRIPTION OF PLATES

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### PLATE 9

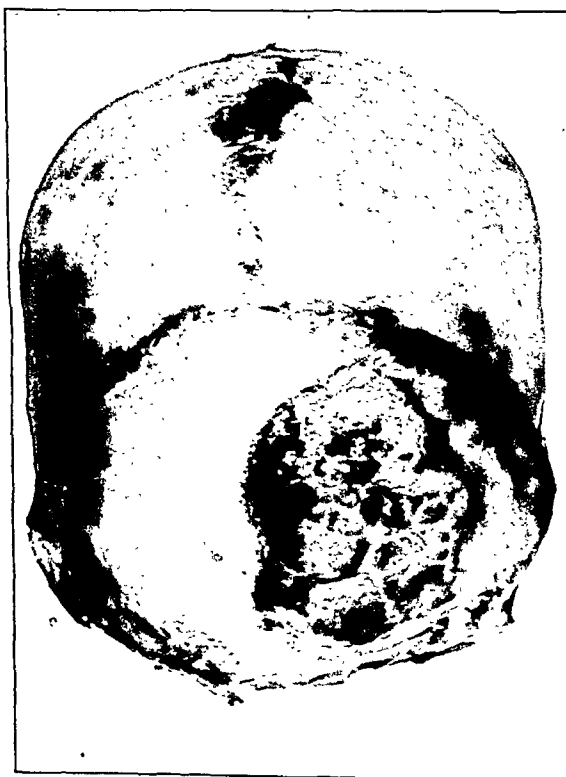
- FIG. 1. Case 1. Photograph of the patient at the time of admission. A slight elevation under the skin in the left superciliary region is indicated by an arrow.
- FIG. 2. Case 1. Photograph of the same patient taken forty-two days later. The primary tumor had been removed: the subcutaneous swellings are prominent.
- FIG. 3. Case 1. The external surface of the calvarium showing a large metastatic tumor mass over the left frontal prominence and smaller masses along the cranial sutures.
- FIG. 4. Case 1. The internal surface of the calvarium showing metastatic tumor masses. The ragged appearance of the tumor is caused by the separation of the calvarium from the dura.



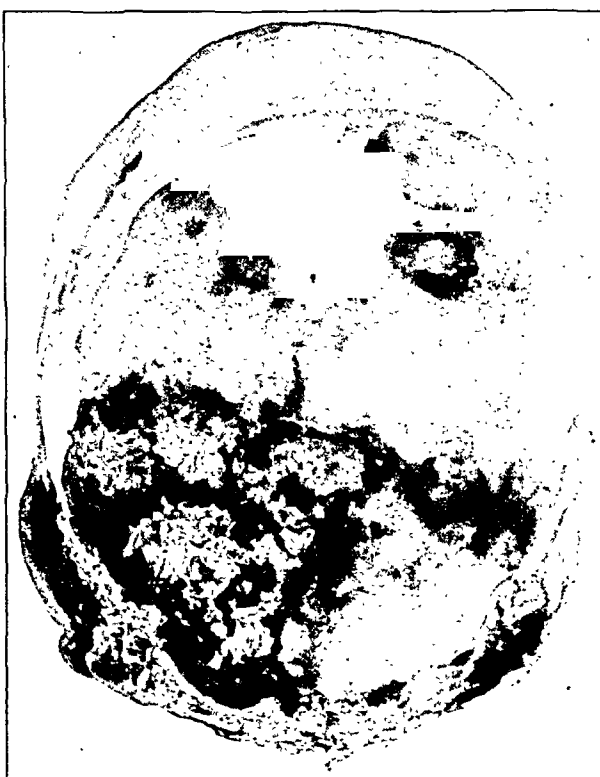
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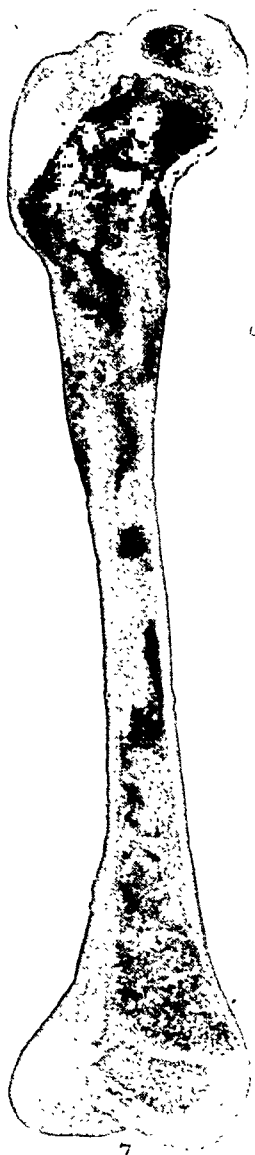
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PLATE 10

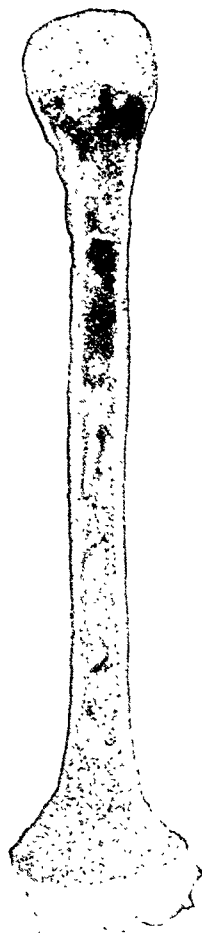
- FIG. 5. Case 1. The external surface of the dura showing one large and many small masses of metastatic tumor.
- FIG. 6. Case 1. Brain, frontal view, showing extensive hemorrhage and two tumor masses in the left frontal lobe. Most of the cerebral veins in the hemorrhagic areas are thrombosed.
- FIG. 7. Case 1. Left femur, showing tumor metastasis filling up most of the medullary cavity. Two masses of the newgrowth are found under the periosteum. The cortex near the neck of the femur has been worn through by the tumor.
- FIG. 8. Case 1. Right humerus showing metastatic tumor in the medullary cavity.
- FIG. 9. Case 1. Longitudinal section of the spinal column showing tumor metastases in the bodies of all the vertebrae. A few rounded tumor masses can also be seen in the pelvis.



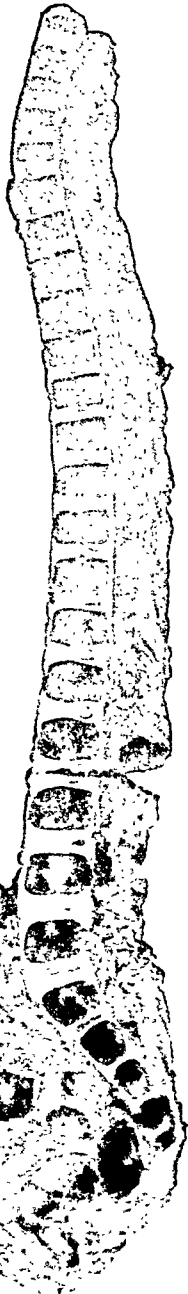
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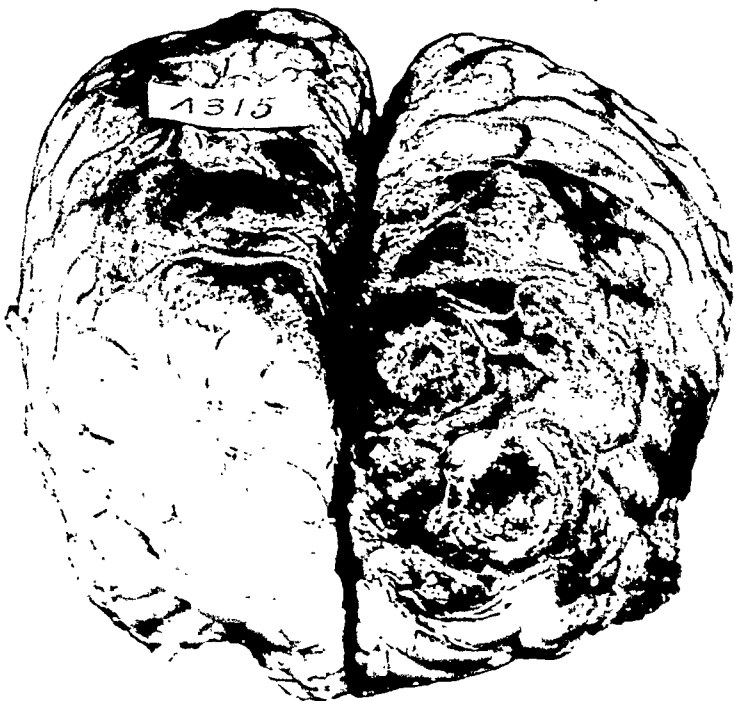
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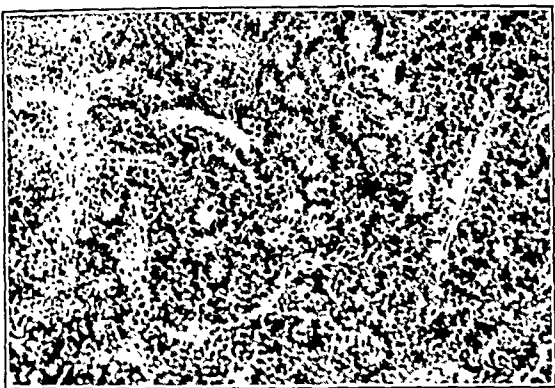
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Neurc-epithelioma (Glioma) of Retina

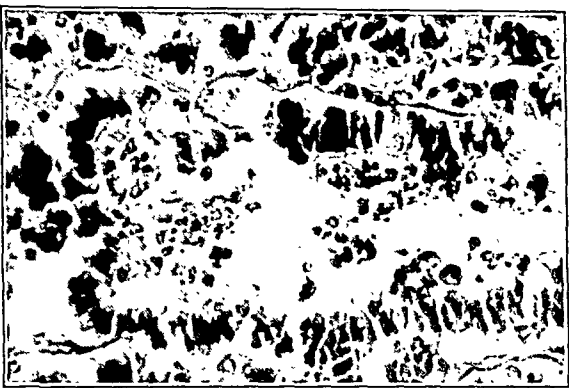


PLATE II

- FIG. 10. Case 1. Primary tumor of the left eye showing rosettes.  $\times 100$ .
- FIG. 11. Case 1. Primary tumor of the left eye showing rows of straight and curved epithelial-like, columnar cells.  $\times 250$ .
- FIG. 12. Case 1. Primary tumor of the left eye showing a row of columnar cells attached to a small band of connective tissue and covered on its surface by a few necrotic cells.  $\times 500$ .
- FIG. 13. Case 1. Primary tumor of the left eye showing rosettes.  $\times 500$ .
- FIG. 14. Case 1. Primary tumor of the left eye showing a curved row of epithelial-like columnar cells with sharp cell borders on one side.  $\times 500$ .
- FIG. 15. Case 1. Primary tumor of the left eye showing invasion of the intra-ocular muscle.  $\times 100$ .
- FIG. 16. Case 1. Metastatic tumor in dura showing the tumor cells better preserved around the blood vessels than those away from them, resulting in what appears to be a perivascular growth.  $\times 100$ .



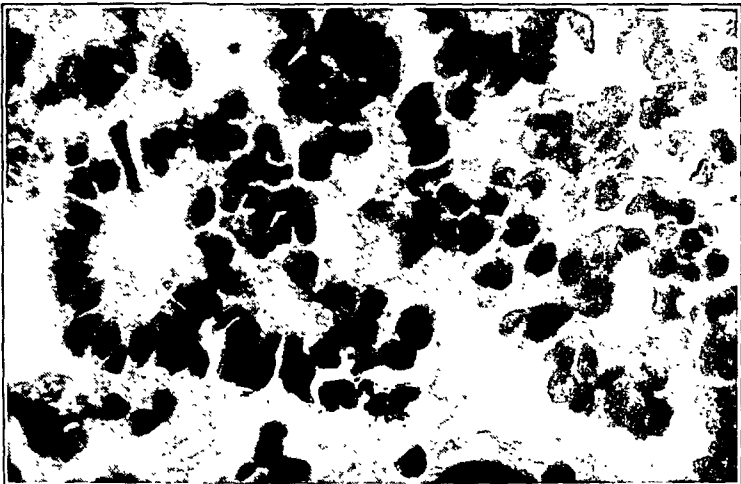
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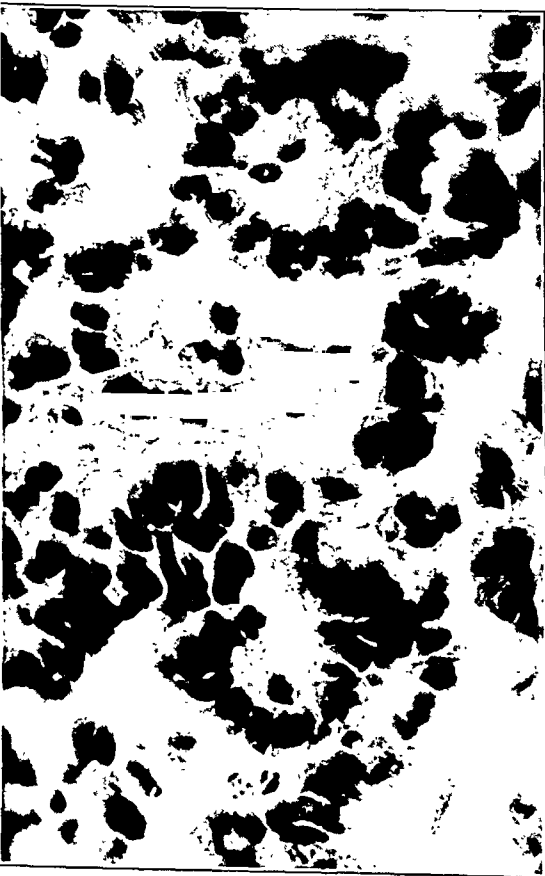
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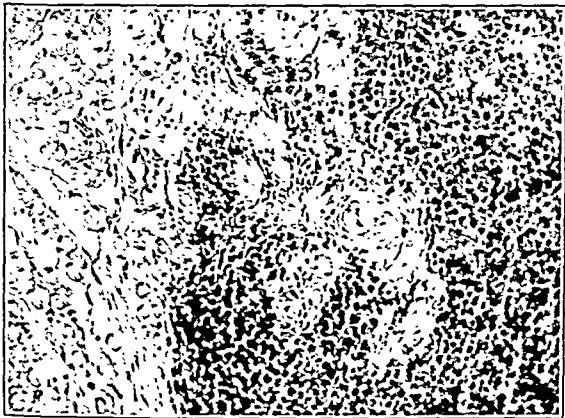
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Hu

Neuro-epithelioma (Glioma) of Retina

PLATE 12

FIG. 17. Case 1. Tumor metastasis in the bone marrow of rib.  $\times 100$ .

FIG. 18. Case 1. Tumor metastasis in the bone marrow of rib, showing two mitotic figures.  $\times 1000$ .

FIG. 19. Case 1. Meninges of spinal cord, showing tumor cells.  $\times 100$ .

FIG. 20. Case 2. Photograph of the patient at the time of admission.

FIG. 21. Case 2. Primary tumor of the left eye, showing the apparent perivascular grouping of the tumor cells following necrosis of those cells farther away from the blood vessels.  $\times 60$ .

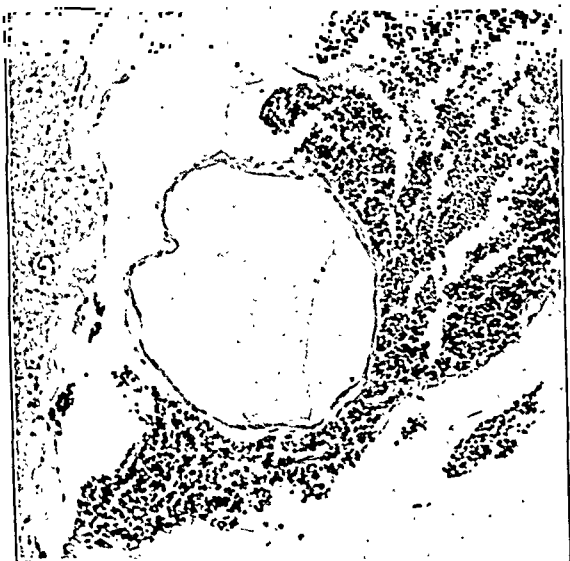
FIG. 22. Case 2. Primary tumor of left eye, showing two mitotic figures.  $\times 800$ .



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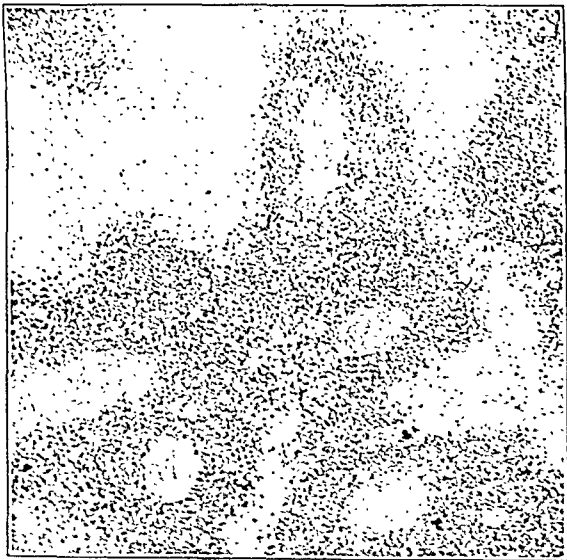
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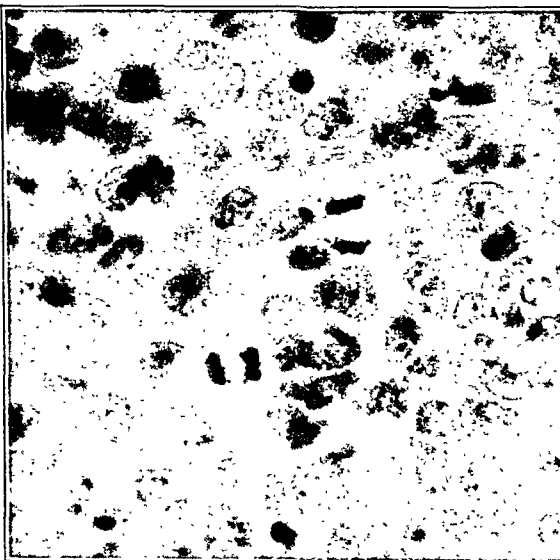
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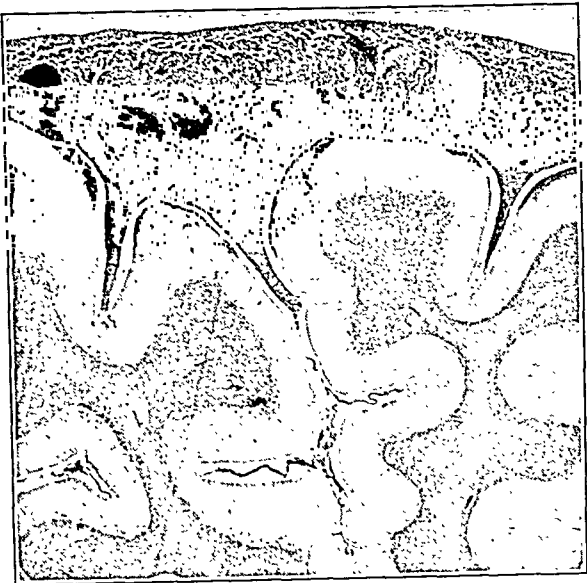
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PLATE 13

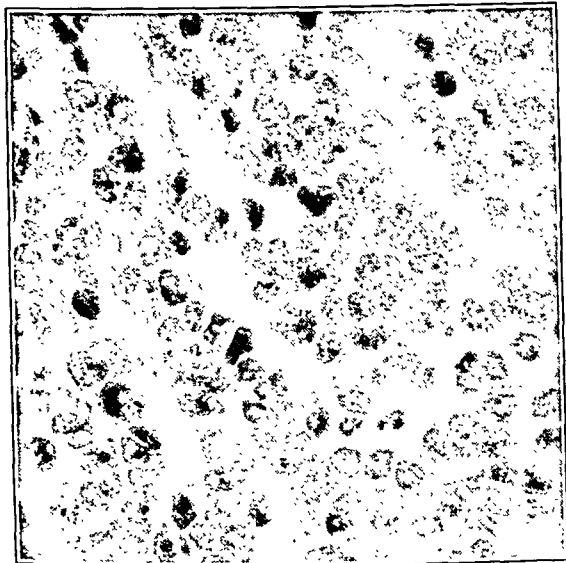
- FIG. 23. Case 2. Gross appearance of cerebellum with metastatic tumor growth on its surface.
- FIG. 24. Case 2. Cerebellum, showing metastatic tumor infiltrating the meninges and invading the cerebellar cortex.  $\times 20$ .
- FIG. 25. Case 2. Metastatic tumor in the meninges of cerebellum.  $\times 600$ .
- FIG. 26. Case 2. Spinal cord, thoracic portion, showing extensive infiltration of the meninges and invasion of the cord by tumor. Sections of cord at different levels show essentially the same picture.
- FIG. 27. Case 2. Spinal cord showing infiltration of tumor cells in the Virchow-Robin's spaces of the blood vessels in the white matter near the anterior horn.  $\times 80$ .
- FIG. 28. Case 2. Anterior nerve roots of the spinal cord surrounded by and infiltrated with tumor cells.  $\times 60$ .



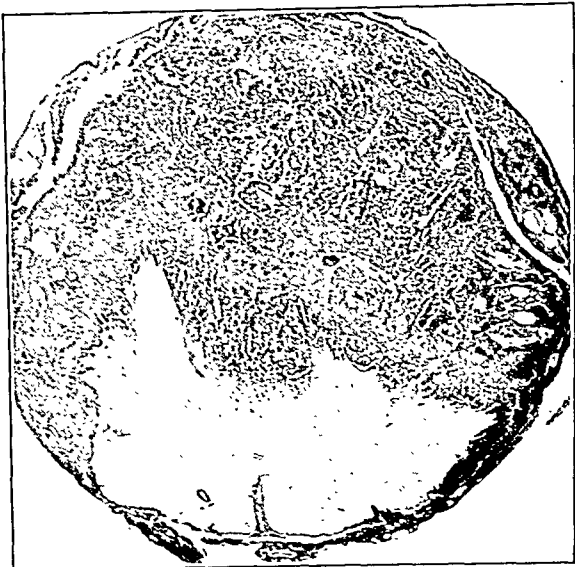
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Hu

Neuro-epithelioma (Glioma) of Retina



## ORIGIN OF THE PERIVASCULAR PHAGOCYTES OF GRANULATION TISSUE \*

F. A. McJUNKIN

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That ameboid mononuclear phagocytes may arise from the vascular endothelium during early embryonic development has been established.<sup>1, 2</sup> Whether phagocytes detach themselves from the vascular endothelium of the adult is controversial. An extensive literature dealing with the problem has accumulated. The vital reaction of cells to dyes and particulate matter, supravital staining methods and tissue culture have been the chief technical methods employed in the investigations of the last few years. As a result of the facts brought out by these newer methods the idea is now prevalent that a majority of the blood and tissue mononuclear phagocytes are derived from the reticular or lymphoid tissue. The cells of this origin are now commonly called monocytes. This view, in my opinion, is correct and it greatly clarifies our conception of the mononuclear phagocytes. Much difference of opinion still exists in regard to the relationship and distribution of the parent reticular and lymphoid tissues. Maximow's<sup>3</sup> extensive investigations led him to think that the lymphocytes themselves were transformed into mononuclear phagocytes (polyblasts). Sabin, Doan and Cunningham<sup>4</sup> by supravital staining with neutral red demonstrated within the monocytic cytoplasm a rosette of dye granules, and they produced evidence that this monocyte is of reticular origin. These authors did not, however, examine in detail the distribution of the reticular tissue in the body. Both Maximow and Sabin and co-authors were of the opinion that the monocyte did not comprise the entire group of mononuclear phagocytes. The latter recognized a second type of cell, the clasmatoocyte, which is derived from the vascular endothelium. Maximow on the other hand is emphatic in his denial that the vascular endothelium of the adult contributes to the group of mononuclear phagocytes, but he expressed the opinion that certain primitive or embryonic cells persist in the extravascular tissues as "resting wan-

\* Received for publication July 29, 1929.



dering" cells and they in response to stimulation become active phagocytes. He thought that the cells lining the capillaries of sinusoidal organs such as the spleen, liver and bone marrow were both actively phagocytic and a source of detached phagocytes. However, he did not think that the lining cells of these blood channels were true endothelial cells but he regarded them as histiocytes and unrelated to true endothelium.

Mallory <sup>5</sup> was the first to recognize endothelium as the fixed tissue source of the mononuclear phagocytes and he has observed and clearly stated <sup>6</sup> that the lymphoid reticulo-endothelium plays an important rôle in the genesis of the group of mononuclear phagocytes named by him "endothelial leucocytes." My earlier investigations <sup>7</sup> convinced me that the reticulo-endothelium (lymphatic and blood-vascular) was the chief source of mononuclear phagocytes in the adult. Until recently, I <sup>8</sup> have entertained the possibility that the peroxydase-reacting phagocytes found in normal human blood may arise in the bone marrow, which is the view held by Naegeli. <sup>9</sup> However, monocytes have the property of ingesting peroxydase granules and these granules for a time retain their ability to react with benzidin.<sup>10</sup> If it be true, as these experiments indicate, that the peroxydase mononuclear phagocytes, or monocytes of human blood acquire their granulation secondarily in a more or less accidental fashion then my conclusion is that all mononuclear phagocytes separate from the reticulo-endothelium, either lymphoid or blood-vascular. In my first experiments <sup>7</sup> with carbon suspensions no distinction was made between endothelial leucocytes of lymphoid origin and those derived from the blood-vascular endothelium since both kinds were marked by the carbon in a somewhat similar fashion. Later experiments <sup>11</sup> with the direct supravital staining of peritoneal exudates, of the blood and of various tissues, and with vital reactions on tissue cultures of lymph nodes <sup>12</sup> introduced new evidence that tended to show a division in the group. The response of the reticular tissue of lymph nodes appearing in tissue cultures to vital tests was a convincing demonstration of the origin of monocytes from this tissue. These experiments indicated that there were at least two kinds of phagocytes one of which was derived from lymphoid reticular tissue. It was felt that evidence of the origin of the second type from blood-vascular endothelium was inconclusive. The earlier observations <sup>7</sup> were made on vascular endothelium of the sinusoidal type,

the true endothelial character of which has been disputed. The capillary endothelium has now been made a subject of further study. Tissue cultures proved of little help since this type of endothelium either does not grow readily, or if these cells multiply they are not easy to identify because of course the structures formed carry no blood. Granulation tissue produced experimentally has been employed for the observations. The first step was to show that the true endothelium of the vascular sprouts is phagocytic, since this has been denied. It was found in experiments already published<sup>13, 14</sup> that when stimulated by trypan blue injections this endothelium is fully as active in the ingestion of carbon as is the sinusoidal endothelium of liver and spleen. In the experiments described in this paper the behavior of the endothelium and the cells in contact with or near it have been observed under varying conditions after having been stimulated and manifesting phagocytosis.

#### PERIVASCULAR PHAGOCYTES OF ENDOTHELIAL SPROUTS MARKED WITH CARBON

Rabbits and rats were used. The granulation tissue was produced by subcutaneous injections of a saturated alcohol-acetone solution of Sudan III or a temporary suspension of the dye in water. Carbon in the form of India ink was injected intravenously (ear vein or tail vein). A number of minor variations were introduced especially by changing the time between the different procedures. As far as the problem now under consideration is concerned it is necessary to select and describe those experiments in which the essential results were seen to best advantage. The tissues were fixed in Zenker's fluid, embedded in paraffin and stained with hematoxylin-eosin.

*Four-Hour Rabbit (Experiment 26):* Two rabbits weighing 1200 and 1800 gm. each were given 2 cc. Sudan III in acetone-alcohol subcutaneously in each groin. On the same day 5 cc. trypan blue (saturated aqueous solution) were injected intravenously. The trypan blue injection was repeated on the second and fourth days. On the eleventh day 4 cc. of India ink (Higgins') were injected intravenously. The ink was injected slowly under ether and in two doses one hour apart. The animals were killed and the groin tissue fixed four hours after the first ink injection. As usual the distribution of the carbon is very irregular with large areas in which the capillaries are practically devoid of ink and others where much carbon is present.

No doubt the ink precipitates in the form of loose masses readily separable, and here and there these enter the arteries to a microscopic area. Such areas were selected for study. Again the structure of the capillaries and small vessels varies. In general where the vessels are older the carbon is situated not only in the elongated cells next the lumen but also in second layer cells of the same shape (Fig. 7). They show no pseudopodia. In other locations granulation tissue is in the process of active formation with the endothelia large and plump and here and there a mitosis. Some of these new capillary sprouts are like the usual textbook description with the narrow pointed endothelia, but much oftener the endothelial cells are of a size and shape suggesting phagocytes rather than lining cells (Fig. 3). The presence of red corpuscles is extremely important in establishing the endothelial character of the structures. Carbon is present not only in the innermost cells but also in cells of similar structure only partly in contact with the lumen or completely removed to the second layer. These cells in size and shape have the irregularity of the so-called sinusoidal type of endothelium. These cells in the ink-marked areas contain carbon particles (Figs. 1 and 2). In other places the new vessels form a reticular network in which cells connect by their cytoplasmic processes. Such cells no doubt later arrange themselves to form vessels of the usual type. These large irregular cells may abut on the capillary lumen on one side while on the other they have reticular processes (Fig. 5). Throughout the experiments there was noted a close resemblance between such structures and the growth of reticular tissue in tissue cultures of lymph nodes.<sup>12</sup>

*Discussion of the Carbon Experiments:* It seems that the carbon enters the cells by phagocytosis and not passively, as claimed by Lang.<sup>15</sup> In the fixed tissue the ink-marked cells are the larger and more irregular ones and not infrequently they are of an appearance identical with detached phagocytes. All stages of separation from the lumina of capillary structure are readily observed (Figs. 4, 5 and 6). Often it is not possible to determine whether the heaping up of endothelium is toward the lumen or outward, but the evidence is strong that leucocytes do not migrate in to assume these various positions in relationship to the lumina of the vessels. It was rather often seen that a group of ink-containing phagocytes were in the vicinity of a vessel with much carbon in its lumen (Figs. 4 and 6). It is not assumed that all such free cells are detached endothelia. If guinea

pigs are injected intraperitoneally with huge doses of India ink the carbon particles within five minutes appear in the sinuses of the sub-sternal lymph nodes. In this experiment the particulate matter unquestionably passes through cell membranes, with phagocytosis playing no part. Such extravascular carbon may of course be taken up by phagocytes already in the tissue spaces, and so the distribution of carbon-marked cells seen in Figures 4 and 6 would be explained. However, the maximum amounts of intracellular carbon in the ink-marked foci are seen where the endothelia are large and often where they are "heaped up" into more than one layer. This it seems should be considered in connection with the structural and functional identity of the endothelia and the detached phagocytes near the lining cells.

#### PERIVASCULAR PHAGOCYTES OF ENDOTHELIAL SPROUTS MARKED WITH TRYPAN BLUE

Full-grown rats were used because they withstood larger doses of trypan blue than did rabbits. The granulation tissue was produced by injection into the groin of 1.5 cc. of an arsenious acid solution made by diluting 0.5 cc. of a saturated aqueous solution of arsenious acid to 6 cc. with 50 per cent ethyl alcohol. Seven days later 2 cc. of a saturated aqueous solution of trypan blue were injected into a tail vein. On the following day 2 cc. of the dye were again injected. The rats were killed two hours, five hours and eighteen hours after the last injection. Inflammatory tissue at the site of the arsenious acid injection shows extensive formation of new capillaries and an inflammatory exudate in which large mononuclear phagocytes predominate. The tissue was fixed in formalin, cut thin for embedding in paraffin after thirty minutes in two changes of acetone and one hour in benzol. After removal of the paraffin from the sections they were mounted in balsam and examined unstained. In this way most of the trypan blue is preserved in the sections which are thin and much better than frozen sections for accurate observation. As a check on the structures, alternate sections have been stained in the usual way with hematoxylin-eosin. In both the five and eighteen-hour animals the dye is sufficiently collected to appear as minute granules in the cytoplasm of both endothelia and detached phagocytes. In the eighteen-hour rats the granules are larger. In the unstained preparations often capillary connections with larger vessels

filled with red corpuscles show the endothelium to best advantage since here the identification of the lining cells is certain. In such locations two to a half-dozen large endothelia may appear in the wall of a capillary, and at the end of five hours and eighteen hours the fine trypan blue granules tend to lie at the ends and external to the nuclei. The distribution of the dye granules is essentially the same as that of the carbon shown in the illustrations. In the same tissue embedded in the usual way and stained with hematoxylin-eosin, dye granules appear only in a few of the extravascular phagocytes where the aggregations of dye are coarse. The tissue used for the carbon experiments was also rapidly embedded and examined unstained so as to preserve the trypan blue. In these the trypan blue was present only irregularly here and there in the form of coarse granules in the cells lining the capillaries, except in animals receiving trypan blue about twenty-four hours before the ink injections, in which instance the picture was more complex with much extravascular dye and a staining of the endothelia like that of the eighteen-hour rats. To test the acute effect of the trypan blue three full-grown rats were injected subcutaneously to produce the granulation tissue, and four days later 2 cc. saturated aqueous solution of trypan blue were injected into a tail vein. The rats were killed five minutes, ten minutes and one hour after the trypan blue injections. In the first two the dye is demonstrable only in the lumina of some of the capillaries where there is a more or less diffuse staining of the corpuscles. In the one-hour rat there are scattered extravascular phagocytes containing blue granules. In some of the endothelia there is a tinge of blue but no granules. Evidently the dye is more or less dissolved whether the sections are frozen and floated on water or rapidly embedded in paraffin, unless sufficient time elapses to permit the cells to concentrate the dye into granules.

*Observations and Discussion of the Trypan Blue Tissue:* Evans and Scott <sup>16</sup> in their study of the macrophage reaction to acid colloid dyes found that the macrophage was the first to respond but that fibroblasts accumulated the dye after chronic administration and finally had a resemblance to the macrophage. In the acute process (up to about two days) the macrophage only contained the dye. It was only after a long time (seventy-five days) that the fibroblast became heavily loaded. By the methods used in my experiments the trypan blue granules become very distinct in endothelia eighteen hours after

the dye administration, although usually the granules are not so large as in the extravascular phagocytes. However, as shown in the illustrations of the carbon distribution where a reticular structure is assumed by the endothelium it often is not possible to be sure whether a given cell is an extracellular phagocyte or definitely part of a capillary. Evans, Bowman and Winternitz<sup>17</sup> found abundant trypan blue in the Kupffer cells of the liver. Here and in other sinusoidal organs some of the capillary endothelium is kept large and active by the normal phagocytic processes in them. In granulation tissue and in most normal tissues the phagocytic property of the endothelium becomes latent unless it is artificially stimulated. In the liver, as in granulation tissue, the evidence now is that some of the perivascular cells are of blood-vascular origin while others are monocytic. Evidence of this in the sinusoidal organs has been presented elsewhere.<sup>11, 12</sup> The capillary endothelium of granulation tissue collects the colloidal dye with a facility equal to that of the Kupffer cells of corresponding size. Since carbon particles may pass through membranes without undergoing phagocytosis the smaller dye particles no doubt do the same. The presence of dye granules in extracellular phagocytes *per se* is not proof of their origin from the vascular endothelium, but the structural resemblance and the positions of the attached and detached cells indicate a relationship between the two.

### SUMMARY AND CONCLUSIONS

1. That the blood-vascular endothelium other than that found in sinusoidal organs has phagocytic properties is confirmed by these experiments. By experimental stimulation the usual type of capillary endothelium assumes the size and shape of the so-called histiocytic endothelium of the sinusoidal organs such as liver, spleen and bone marrow.
2. In the granulation tissue of animals injected with India ink the arrangement of detached phagocytes about the endothelia and the structural resemblance of attached and detached cells are evidence of the identity of the two.
3. The endothelia of the capillaries of granulation tissue segregate trypan blue to form microscopic granules just as the sinusoidal endothelium does. The evidence obtained by the use of colloid dyes, that phagocytes separate from the endothelium of such organs as the

liver and spleen, applies equally to the endothelium of granulation tissue.

4. These experiments indicate that some of the perivascular phagocytes are derived from the vascular endothelium and are not monocytic.

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## DESCRIPTION OF PLATES

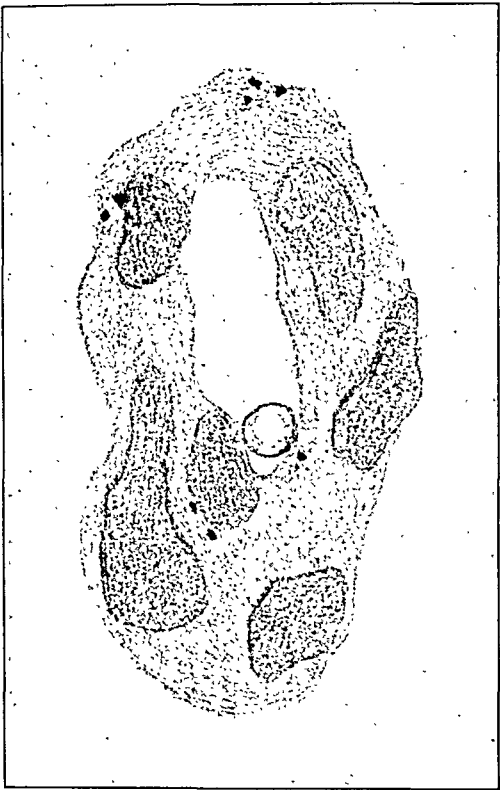
Structures drawn to the same scale with aid of the camera lucida. Tissues from animals receiving intravenous India ink stained with hematoxylin-eosin.

### PLATE 14

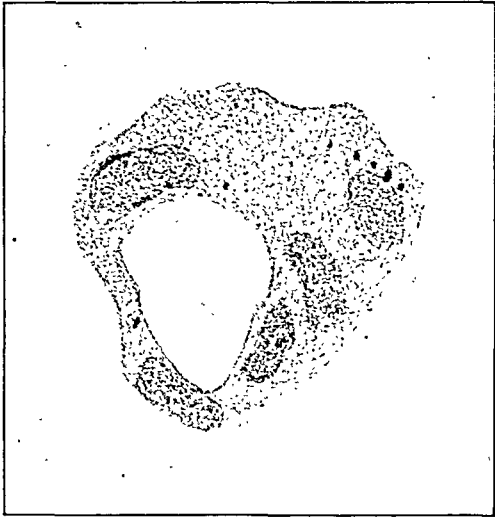
FIGS. 1 and 2. Two capillaries showing the stimulated endothelium with "second row" cells containing carbon. Some of these have only partial contact with lumina.

FIG. 3. Capillary sprout with the second cell which contains carbon in mitosis. The end cell has the ameboid type of nucleus often seen in the endothelia.

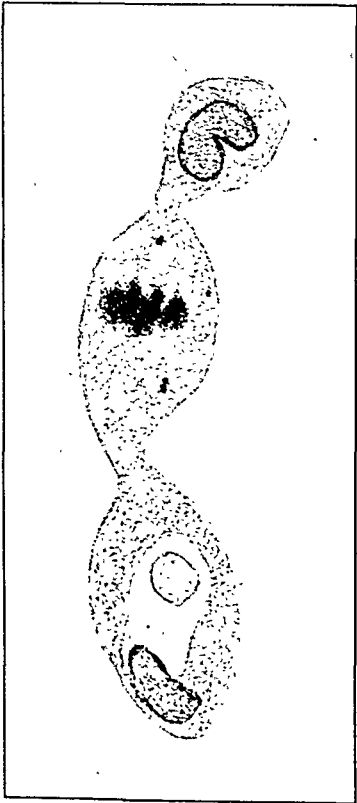
FIG. 4. Much carbon in endothelia and in nearby phagocytes. Often such extravascular carbon within phagocytes is found where the endothelia are large and phagocytic and not where the capillary wall is thin.



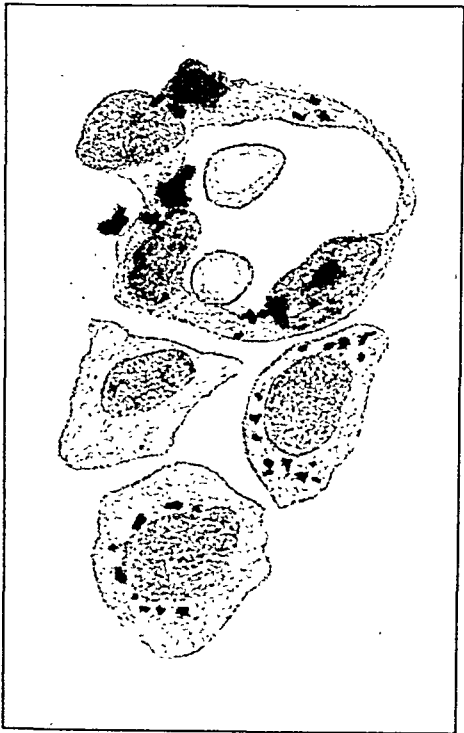
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McJunkin

Perivascular Phagocytes of Granulation Tissue

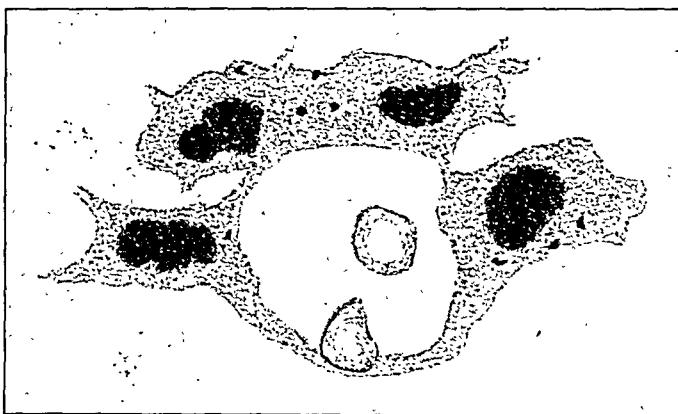


## PLATE 15

FIG. 5. Cross-section of capillary where the endothelia assume a reticular appearance. Such structures are common and the proliferating capillaries have this appearance as frequently as that seen in Fig. 3.

FIG. 6. Two capillaries with carbon in endothelia but none in the phagocytes. Monocytes certainly may be of this size and shape. In the capillary at the left the outer carbon-containing cell has the ameboid type of nucleus.

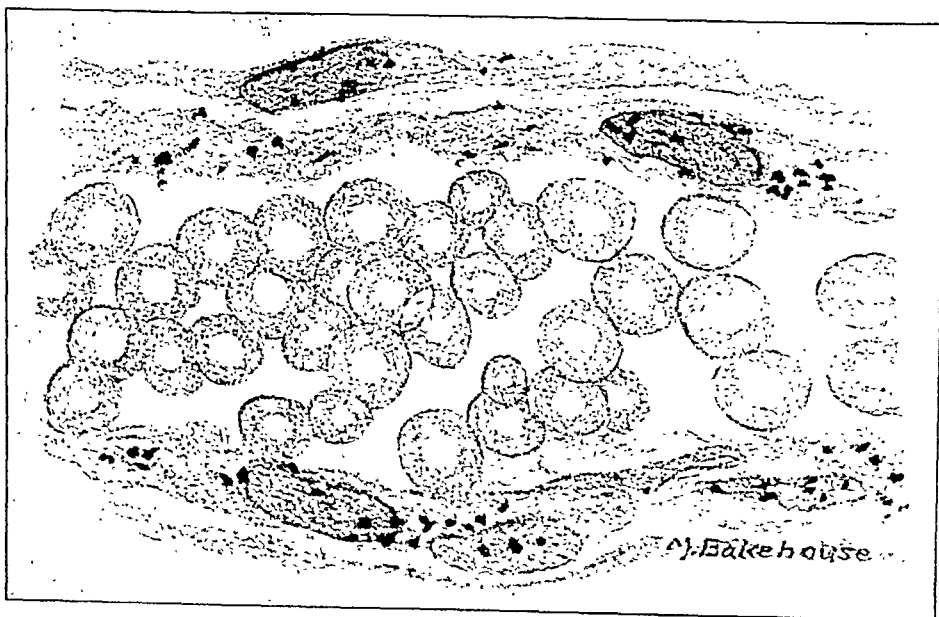
FIG. 7. Longitudinal section of a thin-walled vessel with much carbon in both inner layer and in cells of similar appearance farther out. If the outer cells are of a type different from the endothelia, differentiation is not possible in the sections.



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## METASTATIC INOCULATION OF A MENINGIOMA BY CANCER CELLS FROM A BRONCHIOGENIC CARCINOMA \*

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Boston, Mass.)*

Experimental and clinical investigations on cancer for the last two decades seem to favor the conception that the development of an epithelial malignant disease is largely influenced by immunobiological factors. Apparently not only does the host itself offer resistance to the development of a cancer (natural immunity) but resistance toward the implantation of cancer can be induced experimentally in animals (active immunity). Thus Ehrlich<sup>1</sup> showed that rats with healed mouse cancer are immune to a reinfection, and also that mice with actively growing tumors are mostly immune to secondary implants. Similarly Murray<sup>2</sup> reported that mice with a tar cancer resist a "superinfection" with a second tar cancer, and that a tar tumor will not "take" in a mouse following the extirpation of a previously existing spontaneous tumor. If these observations are correct and also if one is permitted to draw analogies between man and animal, a person with cancer ought to resist the development of a new tumor.<sup>3</sup>

The problem of benign tumors is different in that the condition represents obviously a purely local disease, and for this reason the coexistence in the same person of multiple benign tumors or of a malignant and a benign neoplasm is merely one of casual interest only.

Whether a preëxisting benign tumor provides a suitable soil for the growth of malignant tumor cells and how the latter will behave under the circumstances has been investigated experimentally by Ehrlich as will be told; but it is especially unusual to have the opportunity to study this symbiosis in man.

In the case to be discussed the patient had multiple primary tumors — a leiomyoma of the uterus, and a meningioma in which carcinoma cells were found to be growing.

\* Received for publication September 27, 1929.

## REPORT OF CASE

*Clinical History:* A white woman, aged 57, entered the Peter Bent Brigham Hospital March 14, 1923 with the complaint of pain in the lumbar region extending down the left leg to the knee, inability to walk and weakness of the right hand. The left breast had been removed twenty-five years ago at the St. Luke's Hospital, New York City, for an unknown cause, and she has been well since that time.

The present illness began with a dull, persistent, bilateral lumbar pain five months before admission. Two weeks later the pain extended down the left anterior thigh and left gluteal region. There also was trouble in walking, night sweats from eight to twelve weeks, and hemoptysis for two weeks, a spoonful in amount.

On physical examination the abdomen was rigid and the left lower abdominal quadrant was definitely tender. Several small nodules were palpated on the left side of the cervix uteri.

The blood pressure was systolic 110, diastolic 60. The blood and urine were normal. The spinal fluid showed no pathological changes.

*March 24, 1923.* Roentgen-ray examination of the chest disclosed an area of consolidation in the left base. The thorax was slightly asymmetrical, the left side being less expanded than the right. The left diaphragm was higher than the right.

*March 31, 1923.* The patient was operated upon for a myoma of the uterus. The wound healed slowly owing to a mild infection. The patient coughed up blood.

*May 22, 1923.* A re-examination of the chest showed a diffuse mottling which resembled miliary tuberculosis throughout both lungs. The left lower base was hazy.

*May 31, 1923.* The patient died.

## AUTOPSY REPORT

*Anatomical Diagnoses:* Left bronchiogenic carcinoma with metastases to bronchial lymph nodes, liver, adrenals, bone and brain. Meningioma, infiltrated by cancer. Leiomyoma of uterus. Absence of left breast removed twenty-five years previously. Acute aortic endocarditis. Infarction of spleen and kidneys, and septicemia.

The body was found to be well developed and well nourished. There was an edema of the ankles and legs.

The pleura on the left side was adherent to the thoracic wall. Numerous tumor nodules could be seen in the intercostal muscles and below the periosteum of the ribs.

The heart showed warty, recent vegetations at the aortic valve.

The left lung weighed 580 gm., the right lung 520 gm. The left lung contained a large tumor mass in the lower lobe and in the posterior axillary line just below the interlobar fissure. The two lobes

were adherent by tumor. In this area the lung was contracted, owing to a newgrowth which caused a puckering of the pleura. On section the tumor mass which was 6 cm. in diameter radiated into the lung in various directions. On dissecting down the bronchus it was found that near the tumor the bronchus was definitely roughened and the newgrowth seemed to be present in its wall. Along the course of the bronchi and peribronchial lymphatics numerous tumor nodules could be seen radiating to the root of the lung where very large lymph nodes were found, the largest of which measured 5 cm. in diameter. This extended down the posterior mediastinum to the diaphragm. The nodes at the hilum of the right lung were quite normal. The right lung was literally speckled with small, white, creamy nodules which varied in size from 1 mm. to 0.5 cm. in diameter.

The liver weighed 1,335 gm. and was normally plastic and friable. However, it contained numerous tumor nodules measuring from 2 mm. to 4 cm. in diameter.

Both suprarenals were enlarged and on section thin suprarenal cortex could be seen surrounding grayish tumor masses.

The first lumbar vertebrae were compressed to 1.5 cm. in width and also apparently contained tumor.

*Brain:* The brain weighed 1,300 gm. In the left posterior parietal region was a metastatic nodule which arose at the site of the longitudinal sinus. It measured 1.5 cm. in length and seemed to extend into the secondary sinuses. It was definitely adherent to the dura by clot, and its base seemed in places to have infiltrated the dura. When the skull cap was held to the light it showed areas of increased density more marked than one ordinarily sees in a normal skull, and perhaps evidence of infiltration with tumor.

*Meningioma:* When the calvarium was removed an elevated tumor mass 2 cm. in diameter was found over the right frontal lobe. It was invested by the meninges, being moderately soft and pinkish gray. In gross the appearance of this tumor was that of a meningioma (dural endothelioma).

#### MICROSCOPIC FINDINGS

*Lungs:* The tumor is made up of a columnar epithelium with an oval, vesicular nucleus and a deeply stained nucleolus. The cells have an adenomatous arrangement and are supported by a fine

stroma. In sections taken from the bronchi the tumor shows invasion of all the coats. The circular muscle layer is markedly thickened. The seromucous glands, however, are intact being surrounded by a thick wall of small, round cells. The tumor invades largely the capillaries and veins.

In the suprarenals the tumor closely resembles that of the lungs, while in the liver the stroma is rather abundant, dense and fibrous.

In the brain the tumor is found as small nodules composed of cells identical by their shape and arrangement with those of the pulmonary newgrowth.

*Meningioma:* A cross-section of the entire tumor is studied. In areas where the growth is not invaded by cancer it shows the customary histology characteristic of this neoplastic group. There are numerous psammoma bodies. In places invaded by the malignant epithelial cells (Figs. A and B) the cells of the meningeal newgrowth are dissociated, forming a coarse network. The malignant tumor here is insinuated between the meningioma cells, and the individual cells have adhered to the cellular fibers of the meningioma forming bud-like elevations. Cancerous invasion is more conspicuous at the periphery of the meningioma, however the thick fibrous capsule is not invaded by the malignant neoplasm. In areas where the cancer predominates the meningeal tumor shows a good deal of necrosis.

#### COMMENT

The early occurrence of widespread metastases in bronchiogenic cancers has been discussed by the present writer elsewhere.<sup>4</sup> The significance of this report lies in the fact that the malignant epithelial tumor diffusely infiltrated the meningioma.

Following the successful experiments with the induction of cancer in laboratory animals, Ehrlich conceived the idea of studying the pathogenesis of the so-called mixed tumors in human beings. For that purpose he <sup>5</sup> and Apolant <sup>6</sup> inoculated animals with mixtures of two or three tumors from different germinal layers, like sarcoma and carcinoma, or chondroma and sarcoma.

When a mixture of a carcinoma and sarcoma was inoculated into animals this led to a tumor known as a *carcinoma sarcomatodes* in which the parenchyma was made up of the malignant epithelial cells, while the stroma was sarcomatous. There occurred then an

amalgamation of the two different neoplastic types which resulted in the formation of a new type of tumor.

By injecting into an animal a carcinoma or a sarcoma with a chondroma no amalgamation occurred and both tumors grew side by side keeping their own properties. By mixing, for instance, a chondroma with a sarcoma it was noticed that the benign tumor contained isolated necrotic areas surrounded by actively growing sarcoma. In some areas the tumors were entirely separated so as to represent two distinct neoplasms.

Heiman<sup>7</sup> in a recent study utilized Ehrlich's procedure to bring forward the claimed infectiveness of epithelial malignant tumors. He inoculated rapidly growing carcinomas and sarcomas of the rat in the center of large spontaneous or transplanted fibromas of the breast of other rats. This resulted in the growth of a malignant tumor with, however, a greatly reduced proliferative activity of the carcinoma. The epithelial malignant tumor continued to remain encysted in the center of the benign tumor while sarcomas grew along the track of the needle infiltrating the fibrous tissue and ultimately escaped into the tissue of the host. The benign newgrowth (according to Heiman) seemed to play an entirely neutral rôle even though highly malignant cells were present in the center. This, then, according to the same author, is further evidence against an organism being responsible for the growth of malignant tumors, for it might be expected that if such an organism were present it would stimulate the benign tumor to become malignant.

It will be seen that the clinical case herein reported imitated very closely the experiments of Ehrlich, of Apolant, and of Heiman. The bronchiogenic carcinoma invaded the meningeal newgrowth without being amalgamated with it, and retained therefore its individual characteristics. It grew actively, squeezing out the "host." The carcinomatous cells had split the solid rows of the meningioma giving the impression that they were utilizing its cellular fibrillae as a means of advance.

A few workers are still uncertain whether malignant conditions induced in animals are akin to those seen in human beings. Ribbert (quoted by Lewin<sup>8</sup>) was of the opinion that experimental and human cancer are probably different diseases and that in the problem of epithelial malignant disease no analogies should be drawn between these two species. This of course was denied in many instances.



The case here described demonstrates once more that even "bizarre" neoplastic conditions induced in laboratory animals by Ehrlich and others are often an exact counterpart of what one sees in conditions encountered in the clinic.

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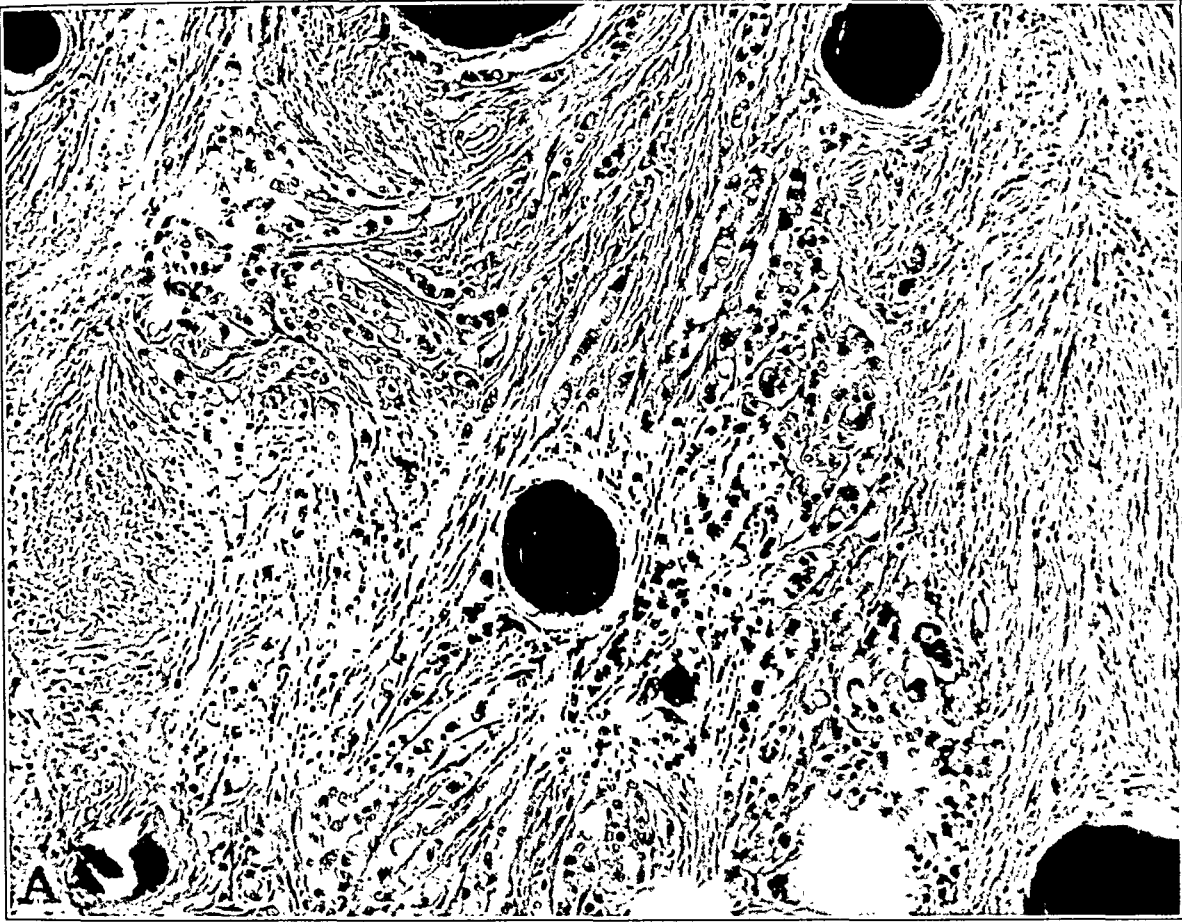
## DESCRIPTION OF PLATE

### PLATE 16

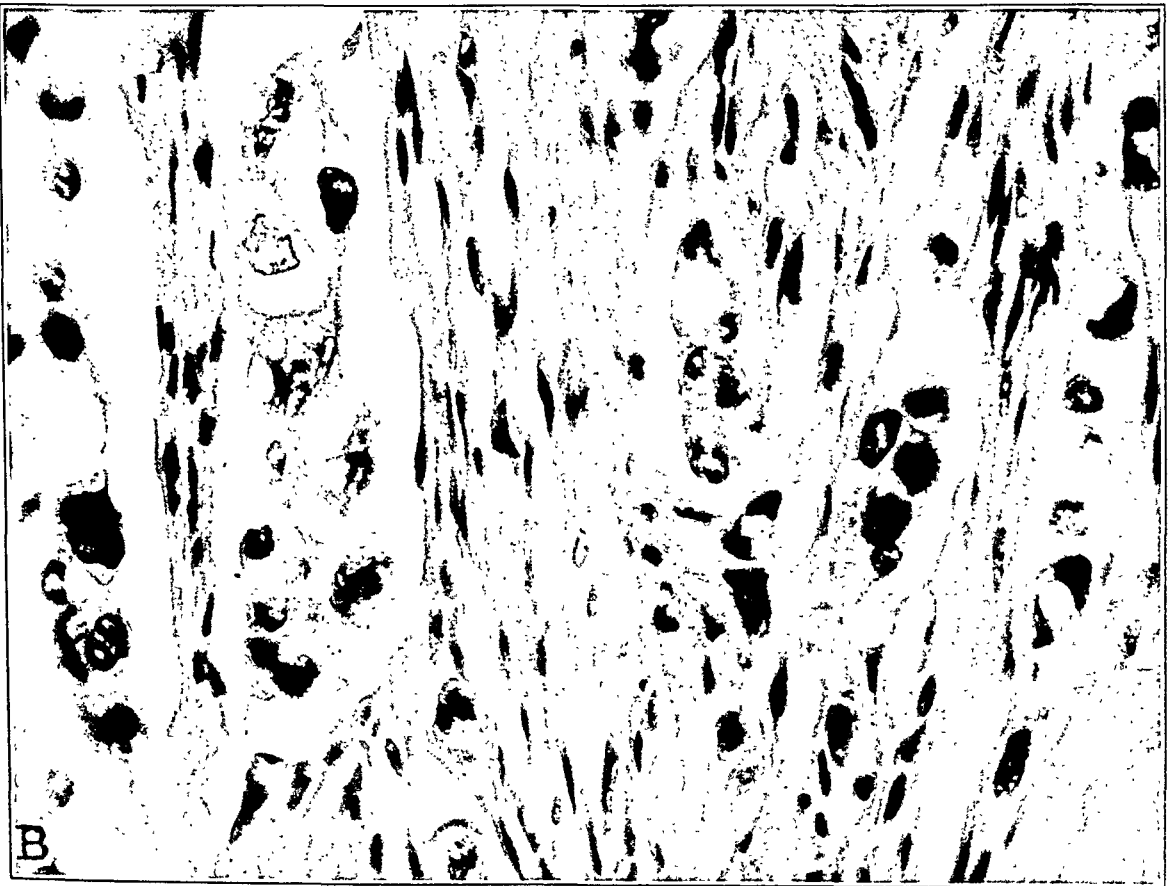
Showing invasion of the meningioma by the cells of a cancer which had originated in the bronchus. Hematoxylin and eosin.

Fig. A  $\times 150$ .

Fig. B  $\times 635$ .



A



B



## STUDIES ON THE SUBMAXILLARY VIRUS OF GUINEA PIGS \*

### II. THE NUCLEAR CELL, NUCLEOCYTOPLASMIC AND INCLUSION- NUCLEAR INDICES OF THE AFFECTED CELLS

GORDON H. SCOTT AND BURCHARD S. PRUETT.

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St. Louis, Mo.)*

In the first paper of this series <sup>1</sup> experiments were reported which showed that the incidence of intranuclear inclusions may be altered at will by experimentally modifying the physiological activity of the submaxillary glands on which the virus acts. Thus, the development of inclusions was found to be greatly aided by stimulation of the gland with pilocarpine, and conversely it was completely inhibited by duct ligation. The latter observation seemed particularly interesting because the operation causes injury and the active formation of young cells — two factors which are believed by Rivers and others to be influential in the promoting of virus action.†

\* Received for publication July 1, 1929.

† To explain this discrepancy Rivers has suggested to one of us (G. H. S.) that the virus may be prevented from entering the gland by the operation of duct ligation and that it would therefore be well to inject it directly into the substance of the glands with their ducts ligated. This was done, and as one would expect, inclusions resulted. But the experiment has no bearing upon the interpretation of my results because the conditions in the glands are wholly different. One set of glands is altered merely by duct ligation while the others have superposed upon this change a variety of other kinds of injury caused by the insertion of the needle, the pressure of injection and the local action of a concentrated suspension of very toxic cellular débris as well as of virus. That the conditions are in no wise parallel is further shown by the difference in the cellular response within the glands. After this direct injection of virus into ligated glands there is much congestion, acute inflammation and destruction of cells, and the inclusions appear in several kinds of cells, as contrasted with the mildness of the reaction and the restriction of inclusions to duct cells which are usually observed in unligated glands after subcutaneous inoculation of the virus. The virus reached the unligated glands presumably by the blood stream; that it was prevented from entering the ligated glands by some unknown barrier which unquestionably permits the free access of the blood necessary to the life of the glands, I do not believe. The ligated glands have a rich blood and lymphatic supply and there is no tendency to sequestration by connective tissue formation.

Cellular hypertrophy is often one of the most distinctive results of virus action. In the lymphocystic disease of fish, for example, the volume of the affected cells may be increased as much as a million times. If we are eventually to understand such changes in volume they must be systematically studied by quantitative methods. The submaxillary virus of guinea pigs is perhaps the best to work with, not only because the intranuclear inclusions called forth are larger than those produced by any other known virus, but also on account of the accompanying hypertrophy of the cytoplasm. In addition to these considerations the cells are more or less uniform in shape and are large enough to permit accurate mensurations. In this contribution an analysis of the volumetric changes in cells caused by the submaxillary virus is given.

### MATERIAL AND TECHNIQUE

The submaxillary glands of four adult guinea pigs spontaneously infected with the virus were selected as material. They were fixed in Zenker's fluid without the usual 5 per cent of acetic acid. Dehydration was carried out slowly and care was taken in all the steps of the technique to avoid undue shrinkage. Serial sections were cut 4 microns in thickness. Some were stained with Giemsa's method, and others with eosin and methylene blue. Because the boundaries of normal duct cells are very indistinct as compared with duct cells modified by the virus, different procedures had to be adopted in gathering data.

*Normal Duct Cells:* Measurements were made by the methods of Jackson <sup>2</sup> and Covell <sup>3</sup> with but slight modification. Sections passing approximately through the center of the submaxillary glands were prepared. The cut sections of the uninfected secretory ducts were outlined with the aid of a camera lucida on transparent celluloid sheets of standard weight and thickness (Eastman Kodaloid No. 3). The magnification used was approximately 1500. Care was taken to keep the focus of the microscope in a single optical plane while making the tracings. Some 700 nuclei and their surrounding cytoplasm were drawn.

The outlines of the nuclei on the celluloid were cut out with a pair of sharply-pointed scissors, and counted. The areas of celluloid representing the cytoplasm and those representing the nuclei were then weighed separately. Since the magnification and the thickness

of the celluloid remained unchanged, the cut surface relations of the nucleus to the cytoplasm and likewise of the nucleus to the cell could easily be ascertained. The former was determined by dividing the weight of the celluloid representing the nuclei by that representing the cytoplasm, and the latter by dividing the weight of the outlined nuclei by the combined weight of nuclei and cytoplasm. The index of the relation between nucleus and cytoplasm and that of nucleus to cell is obviously one of volumes as well as one of surfaces. As each secretory duct was outlined with its nuclei in a single optical plane it is justifiable to assume that the results are an approximation of the volume relations of the cellular parts.

The celluloid weight of the nuclear areas being known, the average actual nuclear area could be ascertained. This was done by dividing the weight of the celluloid by its weight per square centimeter and further dividing this result by the magnification squared. Assuming that these normal nuclei do not vary greatly in size, the average actual cross-sectional nuclear area may be obtained by dividing this figure by the number of nuclei involved. It will now be shown, on the assumption that the nuclei are spheres of radius  $R$ , how the volume of a nucleus can be determined from this area which is denoted by  $a$ . It is desired to determine the volume  $V$  of a sphere, the average value  $a$  of its cross-section being known.

The average area of the cross-section of a sphere is the cross-section of a cylinder which has a height equal to the diameter of the sphere and the same volume as the sphere. Let the cylinder "representing" the sphere have volume  $V$  cross-sectional area  $a$ , and height  $h$ , such that  $h = 2R$ .

According to the rules of solid geometry the volume of a cylinder is equal to the cross-sectional area multiplied by the height ( $ah$ ) so that in our case  $V = 2aR$ . As this is also the volume of the sphere which is given by  $\frac{4}{3} \pi R^3$ ,

$$\text{we have: } 2aR = \frac{4}{3} \pi R^3 \quad \text{or} \quad a = \frac{2}{3} \pi R^2.$$

Here  $a$  is the *known* average cross-sectional area and  $R$  is the radius of the sphere whose volume is to be found. From the last equation it follows that:

$$R^2 = \frac{3a}{2\pi} \text{ and } R = \sqrt{\frac{3a}{2\pi}}$$

The volume  $V$  can now be determined by eliminating  $R$  from the relation  $V = \frac{4}{3} \pi R^3$  with the result:

$$V = \frac{4}{3} \pi \left( \sqrt{\frac{3a}{2\pi}} \right)^3 = \sqrt{\frac{6}{\pi}} \sqrt{a^3} = 1.382 \sqrt{a^3}$$

Having derived the formula  $1.382 \sqrt{(\text{average area})^3}$ , the average nuclear volume is readily calculated by simple arithmetic procedures.\* The mean cytoplasmic volume may be computed by multiplying the average nuclear volume by the figure in the nucleocytoplasmic ratio (area of cytoplasm  $\div$  area of nucleus) representing the cytoplasm to which result the volume of the nucleus is added for an estimation of cell volume.

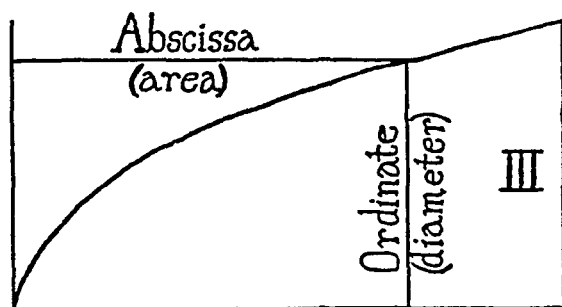
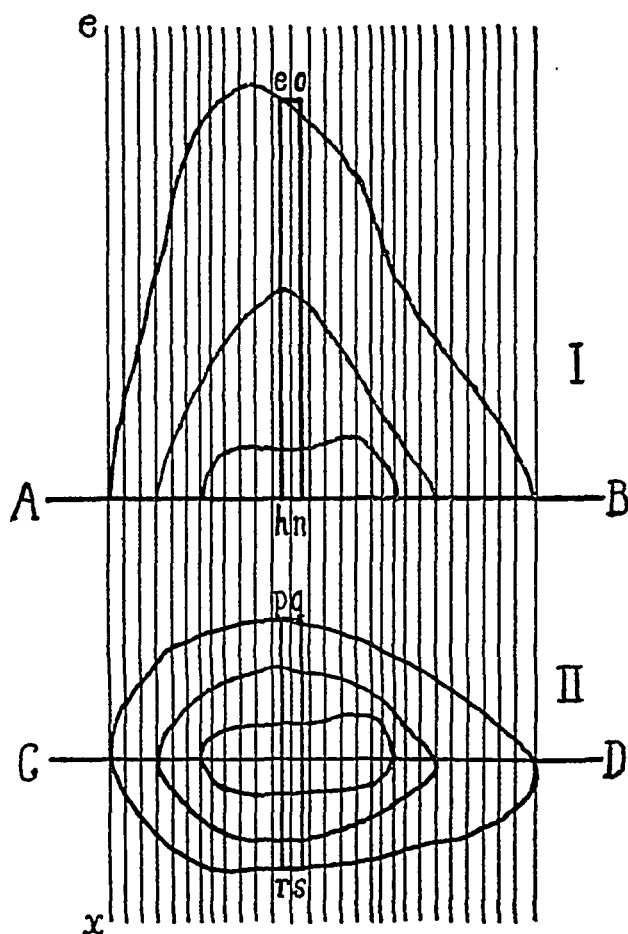
It should be pointed out in connection with this means of determining nuclear and cell volumes that the more nuclei measured, the greater the accuracy of the results. This statement is easily capable of experimental proof by drawing upon ruled paper a circle of known radius and computing the volume of a sphere of the same radius by the usual means ( $\frac{4}{3} \pi R^3$ ). Then determine the areas of sections through the sphere at ten equidistant points along the diameter of the circle and compute the volume of the sphere by the formula  $1.382 \sqrt{(\text{average area})^3}$ . It will be seen that this result is rather far from the true value. If another set of areas is determined at twenty points along the diameter the result will be considerably closer and if forty or fifty readings are made the results approach each other with some exactitude.

Direct measurements of nuclei of the normal duct cells were made by means of a filar-wheel micrometer at the same magnification. In all, 450 of these measurements were made and used as a check on the calculated radii and on the computed nuclear volumes.

*Infected Duct Cells:* It is evident that the method used for the normal cell volume is not applicable to infected cells, because the latter do not occur in compact groups but are isolated and surrounded by seemingly normal cells.

After trying out several possible methods the one used by Boyden<sup>4</sup> for determining the volume of the gall bladder was finally adopted. One fundamental assumption is necessary to its use in this connection, namely, that the object be cylindrical or spherical about a central axis. Fortunately infected cells are generally of this shape (see Fig. 1), and care was taken to select those which conformed as nearly as possible to this specification. As an additional precaution wax models of the cells, their nuclei and inclusions, were made in several instances to serve as a check on the cell shape. The method

\* The authors are deeply indebted to Doctor Vladimir Rojansky, of the Department of Physics, for helpful criticism and aid in the solution of certain mathematical problems involved in this investigation.



Text-Figure 1

A diagrammatic representation of the method used in determining the volume of the cell, nucleus and inclusion in the infected cells.



finally adopted to determine cell volume was somewhat laborious and is dependent to a certain extent upon the mechanical skill of the observer; but statistical analysis of the data thus secured showed that it was dependable and productive of accurate results.

Camera lucida tracings of the cells, their nuclei and inclusions, were drawn at a magnification of 2060 diameters.

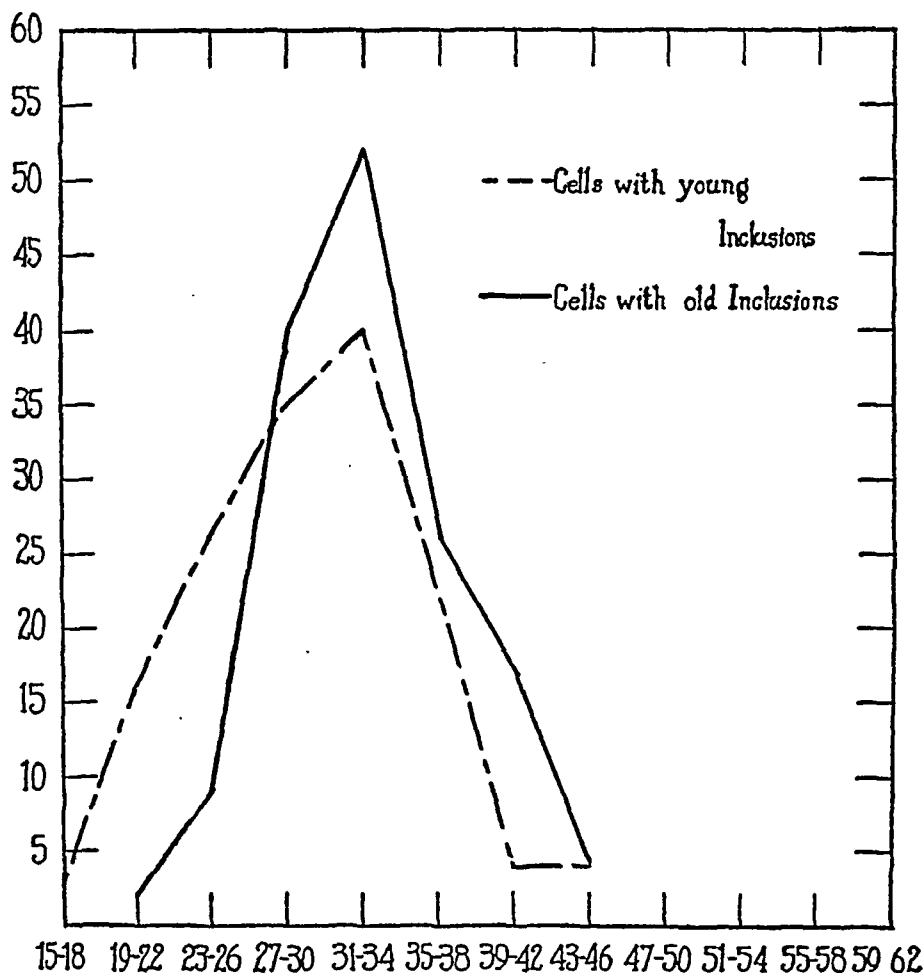
In Text-Figure 1 is a camera lucida tracing of a cell, the nucleus of which contains a mature inclusion body, bisected longitudinally (II). The line  $AB$  was drawn parallel to the long axis of the cell  $CD$ . A series of perpendiculars which cut the cell into a number of cylinders of unknown height were erected to the line  $AB$ . Care was taken to erect the first perpendicular so that it just touched the edge of the cell. The same precaution was observed in passing these lines through the nucleus and the inclusion. With a divider the various diameters of the cell were measured and a distance numerically equal to the area of a circle with the same diameter was laid off from the base line  $AB$  on each perpendicular. To facilitate the process a graph (Text-Figure 2) was constructed so that the abscissa of any point was equal to the area of a circle with a diameter of that of the ordinate.

As an example let the diameter  $pr$  be determined with the divider and the distance be made to fall from the base line to the curve at some point. Then let the length of the abscissa from that point on the curve be determined with the divider. This distance may then be laid off from the base  $AB$  as the line  $eh$ . When this process has been completed for all segments of the cell, a smooth curve is drawn through all the points. The same procedure is followed through for the nucleus and for the inclusion. It is apparent from elementary calculus that the area enclosed by the line  $AB$  and by each of the three curves drawn is equal to the volume of the magnified cell and its respective parts. The areas were measured with a planimeter, and, in order to reduce the term obtained to the actual volume, were divided by the magnification cubed ( $2060^3$ ).

In theory the method is simple. The shape of the cell is considered to be approached by a series of circular discs. Let  $pqrs$  (II) represent one of these discs as seen from the edge. Then the height  $eh$  of the rectangle  $eohn$  (I) is numerically equal to the area of the base of the disc  $pqrs$  and the width of the rectangle is equal to the height of the disc. Since the volume of any cylinder is equal to the area of its base times its height, then the area of rectangle  $eohn$  is equal numerically to the volume of the disc. The integrated volume of  $x$  number of these discs is therefore approximately numerically equal to the magnified volume of the cell and its parts.

Further considerations of the theory of this method are given by Boyden <sup>4</sup> in describing its application to the human gall bladder in the living subject.

In addition to the quantitative methods for cell study, micro-chemical procedures were used to obtain information concerning changes other than volumetric. Sections of infected glands from



Text-Figure 2

A point-to-point frequency graph which illustrates the distribution of the observations on the nuclear cell indices of the cells with early and late inclusion bodies. Ordinates: number of observations at given values. Abscissas: nuclear cell indices.

young and old guinea pigs were prepared and stained for the demonstration of mitochondria. The Feulgen reaction for thymonucleic acid was applied to salivary glands according to the directions given by Cowdry.<sup>5</sup> Tests for potassium were made by the Rohdenburg and Geiger<sup>6</sup> modification of the familiar Macallum<sup>7</sup> technique.

hain's iron hematoxylin and eosin, Unna's methylene blue, Löffler's methylene blue, Gram's stain, carbol fuchsin and methylene blue, Giemsa's stain and phenol iodine green. Routine hemalum and eosin, and eosin-methylene blue staining were found to be sufficient for diagnostic purposes and for examination of the morphology of the infecting organism. The special stains were of value in securing photographs of certain structural details.

Upon examination with the lower powers of the microscope the specimen showed the structure of an inflammatory polypus with a moderately vascular, edematous, connective tissue stroma. In the stroma there were many infiltrating cells, chiefly lymphocytes and plasma cells but with a few polynuclear and polymorphonuclear leucocytes. As compared to the usual type of edematous nasal polypus occurring in chronic hypertrophic rhinitis, the stroma was denser and showed more fibroblastic proliferation, less edema and but very few eosinophiles. Nowhere was the cellular infiltration distinctly purulent. The most striking feature was the presence of the characteristic parasitic cysts, each a single organism, in various stages of development. Averaging about 100 microns in diameter, these cysts were closely placed throughout the polypus so that more than one-half the area in any field was covered by them (see Fig. 1). Each cyst was surrounded by a doubly-contoured chitinous-appearing capsule which showed concentric lamination when favorably stained, and especially in the more mature individuals. The younger organisms, when cut through the central part, showed a well stained karyosome about which there were aggregated nutrient granules and vacuoles containing materials staining in part with basic, and in part with acid, stains (see Figs. 2 and 3). Some of the cysts showed irregularity of form apparently resulting from mutual pressure. By some of the earlier authors this deviation from spherical form was thought to indicate ameoboid movement.

Accepting the developmental life history of *Rhinosporidium seeberi* as worked out by Ashworth, practically all stages can be illustrated from our material. There is no evidence presented which is contradictory to the results of his investigation. The early development of the trophic stage can be illustrated by a parasite measuring but 8 microns in diameter, but already showing the doubly-contoured wall (Fig. 4). No trophic granules are present. By the time the parasite reaches a cyst-size of 60 microns the trophic granules

## HISTORY OF CASE (A.D.R.)

The patient was a male engineering student, aged 26 years, who was born at Clarksdale, Missouri, and who had never been outside of the United States except for three days spent in Canada. He had had no close association with any foreign-born person. He had, however, lived on a farm for twelve years, from the age of 6 to 18 years. During this period he was undoubtedly playing and working in close contact with horses and cattle.

His mother and father were American-born and they, as well as five brothers and two sisters, were living and in good health. No other member of the family had experienced a similar condition.

The patient had first noticed the lesion in his nose in February, 1926, following an injury received while wrestling during the preceding month. At that time the nose was tender and painful to the touch but no history of unusual discharge, foul odor, or bleeding was obtained. Shortly thereafter the right side of the nose was operated upon for nasal polypi by Dr. J. M. Brown of Maysville, Missouri. No further trouble was noted until after a second injury while boxing in February, 1928. Progressive obstruction of the right side of the nose developed and had become almost complete at the time of a second operation in May, 1929.

When the patient presented himself for examination on June 22, 1929, there was found a polypoid growth of the nasal mucosa attached to the right septal wall about 1.5 cm. within the vestibule of the naris. There was a marked induration of the base and area of attachment and some granulation tissue formation. The greater part of the polyp was a grayish, semitranslucent rounded mass with a slight amount of denser vascular tissue extending into it at the base. The polypoid structure was removed with the snare and the base cauterized. Recovery was uneventful and there was no indication of recurrence four months after removal.

## PATHOLOGICAL DESCRIPTION

The specimen when received was a small rounded mass looking not unlike an ordinary nasal polypus. It was bisected before being impregnated in paraffin and revealed no feature attracting attention. It was not, however, examined closely as would have been done had its true nature been suspected.

The Indian physicians believe that the polypoid rhinosporidial growths can be recognized as such by naked-eye examination. The smaller polypi are said to be very similar to a raspberry (*Tirumurti*<sup>12</sup>) in form and color. These growths are friable, tearing easily and bleeding severely when torn. Various writers have described a central, branching, vein-like supporting stroma. The larger parasites within the tissue are visible to the unaided eye as white spots of pin-point size.

Our material was examined first as stained routinely with hemalum and eosin. Later, additional sections were stained with Heiden-

*Geographical Distribution:* Since there seems to be morphological identity on the part of the causal organism in all cases described as due to *Rhinosporidium kinealyi* or *Rhinosporidium seeberi* we must conclude for the present, at least, that but one species is concerned with the production of the disease in question. The geographical distribution then becomes all the more remarkable for its apparent discontinuous character. Most of the cases have been found in southern and western India and Ceylon. Then we have the early group of three cases from Argentina and finally the three scattered cases from the United States. The first of these patients had never been far from Memphis, Tennessee; the second had spent most of his life in Illinois, but had been in Florida and Oklahoma; our present patient was born in Missouri and had spent the greater part of his childhood on a farm, but was in Michigan at the time the polypus was removed. In view of such a wide but discontinuous distribution, it seems probable that infection with *Rhinosporidium seeberi* is much more common than the few reported cases would seem to indicate. Clinically, the condition could not possibly be recognized except by those thoroughly familiar with it. Only microscopic examination of the tissue removed can bring about its recognition. Can it be that many cases considered ordinary polypi and not examined microscopically are due to this infection? Our own experience seems to discredit that explanation. We have routinely examined all nasal polypi removed at the University Hospital as well as many others sent to this laboratory from other sources. Although the total reaches many hundreds no other example of this infection has been found. The peculiar geographical distribution could be readily explained if it were found that some lower animal is a frequent carrier of this organism and man but an occasional recipient. From case reports it is evident that many of those suffering from this infection have lived on farms or have been in close contact with farm animals or the products of farm animals, such as hides. It is not at all certain that this is true of all. It is of great interest that a similar condition has been described for the horse in South Africa, the causal organism having been named *Rhinosporidium equi* by Zschokke.<sup>19</sup>

*Age and Sex Incidence:* *Rhinosporidium seeberi* infections occur particularly in young men, although no age is exempt. It has been found in boys as young as 10 years and in men 60 years of age. It is remarkable that not one of the forty or more reported cases has been

are abundant and the nucleus undergoes changes preparatory to its first division (Fig. 5). Shortly thereafter the chromatin is partially extruded from the karyosome in the form of coarse threads (Fig. 6). With further nuclear divisions the parasite increases in size and the envelope is thickened by the addition of concentric laminae on its inner surface so that it now becomes very distinctly striated. This is especially marked about one region where an annular thickening marks the site of the future pore for the discharge of the mature spores. The nuclei have now considerably increased in number and are scattered through the cytoplasm in which there is still abundant trophic material. In a later stage the nuclei multiply until they pack the interior of the cyst and the cytoplasm becomes concentrated about them and apportioned to them, completing cytoplasmic division (Figs. 6 and 7). With the maturation of a portion of the spores the parasite, which may now be termed a sporangium, reaches a size of 200 to 300 microns. The pore is now ruptured and the spores escape (Fig. 10). Such of the spores as are mature develop a limited number of spherical granules, 4 to 16, which are apparently distinct from the centrally placed karyosome and which according to recent investigations are not reproductive bodies (Fig. 11). With the escape of the mature spores the cycle is complete as far as is now known. It seems probable that such spores can proceed to the development of the early trophic stage directly, although full evidence in respect to this stage is lacking.

The emptying and collapse of the ripe sporangium is followed by a local proliferative reaction of foreign body type. The remains of the wall and such immature spores as are present may be included within multinucleated giant cells which persist for some time, giving the usual type of foreign body pseudotubercle formation. Foreign body giant cells have not been observed in the granulation tissue of this infection except in connection with the disposition of the remains of ruptured sporangia as here described.

#### GENERAL CONSIDERATIONS

*Systematic Position of the Organism:* As a result of reworking the morphology and probable life history of *Rhinosporidium seeberi*, Ashworth found it necessary to assign it an entirely new systematic position. He believes it to belong to the lower fungi and not to the sporozoa, and tentatively places it in the suborder Chytridineae.

*Clinical Characteristics:* Polypoid or papillomatous inflammatory newgrowth is the general characteristic of this condition. In the nasal tract this growth tends to become obstructive. Clinically it exhibits a marked tendency to bleed. After the usual form of operative intervention it practically always recurs, so that most patients give a history of repeated operations before the true nature of the process is recognized. There is no evidence of a generalized hematogenous dissemination in the reported cases.

*Treatment:* Wright was successful in the treatment of his case of *Rhinosporidium* infection of the conjunctiva with 2 per cent tartar emetic dropped into the eye three times a day. He therefore suggested similar applications for the nasal tract, possibly in the form of a daily spray and a weekly pack of the involved nostril. Usually the method of choice will be operation, but this should include the entire area of origin and not simply removal of the polypoid mass with snare or scissors. Patients should be advised of the likelihood of recurrence and of the desirability of frequent re-examinations.

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in a female. It must be that the male is exposed to the infection in some manner which is not shared by the other sex. Wright<sup>17</sup> found three cases involving the conjunctiva in boys in the Civil Orphan Asylum in Madras. Not one of the girl inmates living under precisely the same conditions was affected. Both groups used the same artificial swimming pool, in which the water was changed once in two or three weeks, and the same dusty playground.

*Anatomical Distribution:* While the earlier examples of infection with *Rhinosporidium seeberi* were all of the nasal and nasopharyngeal area, subsequently described cases have extended the anatomical distribution to include the aural canal, the conjunctiva and lachrymal sac, the uvula and the penis. Tirumurti<sup>12</sup> predicted its eventual recognition from the external auditory meatus, mucous membrane of lips, cheek and tongue, larynx, vagina and rectum. Since in the nasal tract infection occurs in areas covered by either columnar or squamous epithelium, there seems to be no good reason why this prediction will not be realized. With knowledge of the wider distribution, the designation *Rhinosporidium* has become much less appropriate.

*Mode of Infection:* Nothing is known with certainty about the mode of infection and of transmission. The possibility of an animal host, probably among the larger farm animals, has already been mentioned. All efforts at experimental animal inoculation have given but negative results. Monkeys, guinea pigs and rabbits were tried by Ingram,<sup>9</sup> guinea pigs again by Tirumurti,<sup>12</sup> monkeys by Wright<sup>16</sup> and Cunningham, and mice, rabbits and guinea pigs by Rettie, working with the material from Ashworth's case.<sup>2</sup> Likewise, with one possible but unproved exception, all attempts to grow this organism have been unsuccessful. The possibility of contact infection is suggested in the histories of several patients who had been associated with others having a similar condition. A majority of the reported cases give no evidence of association with other cases. Finger-borne infection cannot explain those examples occurring well back in the nasal tract, the nasopharynx and the uvula. If the organism is transmitted in dust or water, an assumption in accord with its anatomical distribution, it is difficult to understand its limitation to males, as Ashworth points out. The occurrence of auto-inoculation is clearly shown by the development of additional polypoid growths in nearby but new sites.



## DESCRIPTION OF PLATES

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### PLATE 131

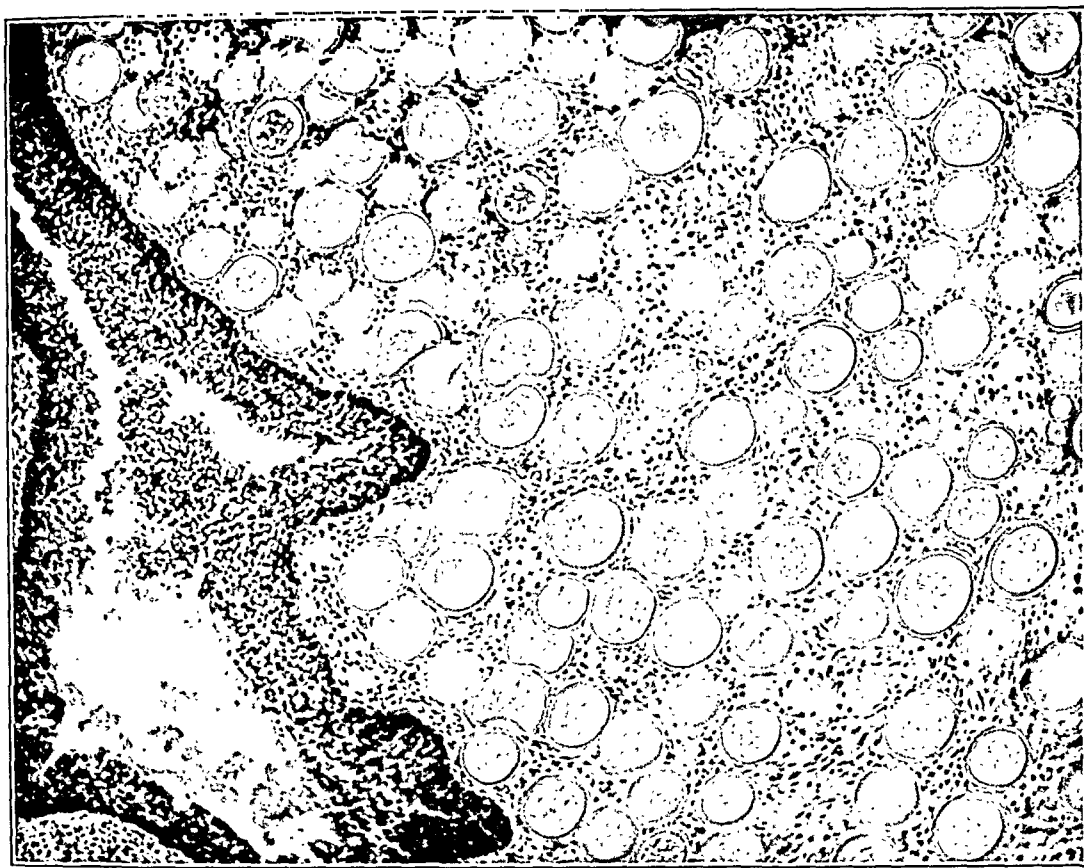
- FIG. 1. Rhinosporidial nasal polypus. The closely approximated parasitic cysts fill the field, leaving but little inflammatory stroma visible.  $\times 90$ .
- FIG. 2. Higher power view showing the cellular stroma between the parasites. The organism in the middle of the field is cut so as to show the central karyosome and the surrounding nutrient substance.  $\times 370$ .

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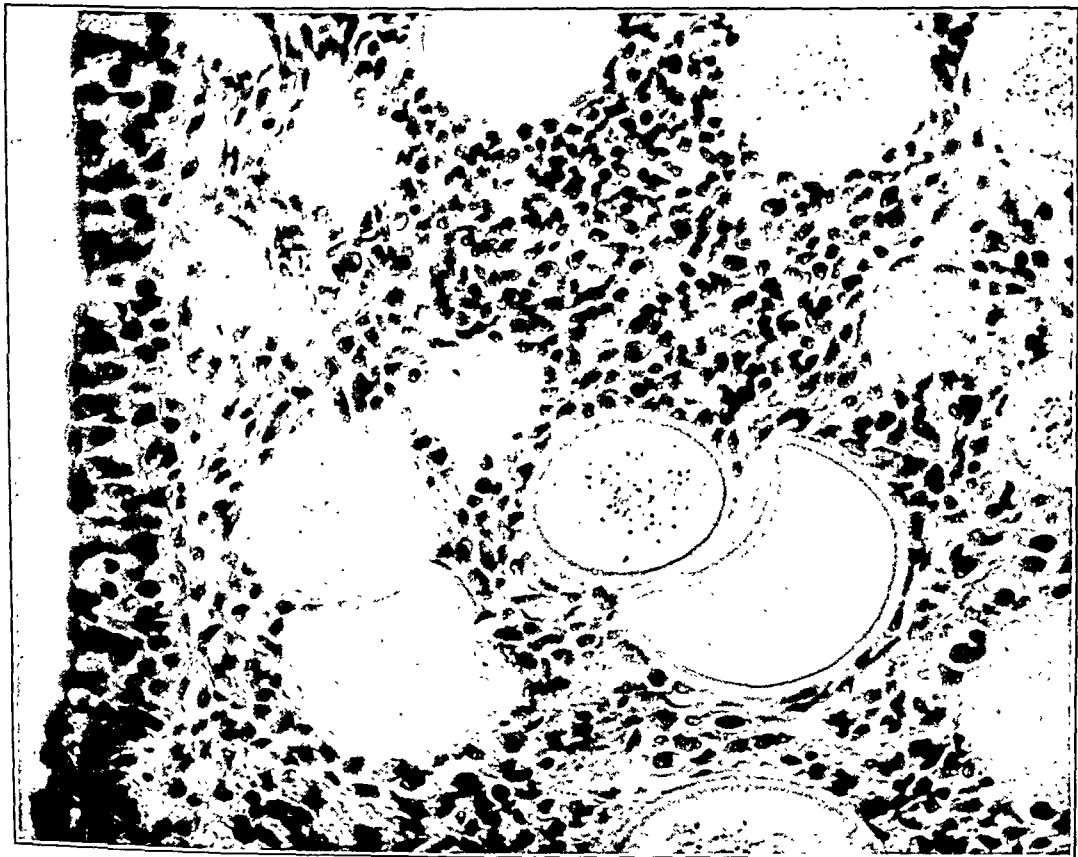
PLATE 132

FIG. 3. Cellular stroma with fibroblastic proliferation. Two parasites show the central karyosome in the midst of the granular nutrient substance.  $\times 370$ .

FIG. 4. Very early trophic stage of *Rhinosporidium seeberi*. Cyst 8 microns in diameter. The two-lobed arrangement of the chromatin is not constant.  $\times 1500$ .



1



2

## TREATMENT OF DATA

It was found inadvisable to attempt to treat the ordinary expression of nuclear and cytoplasmic relations, the nucleocytoplasmic ratio, by statistical methods. To avoid this difficulty the following expressions of relation were used:

$$(a) \text{ Nucleocytoplasmic index} = \frac{\text{Volume of nucleus}}{\text{Volume of cytoplasm}} \times 100,$$

$$(b) \text{ Nuclear cell index} = \frac{\text{Volume of nucleus}}{\text{Volume of cell}} \times 100,$$

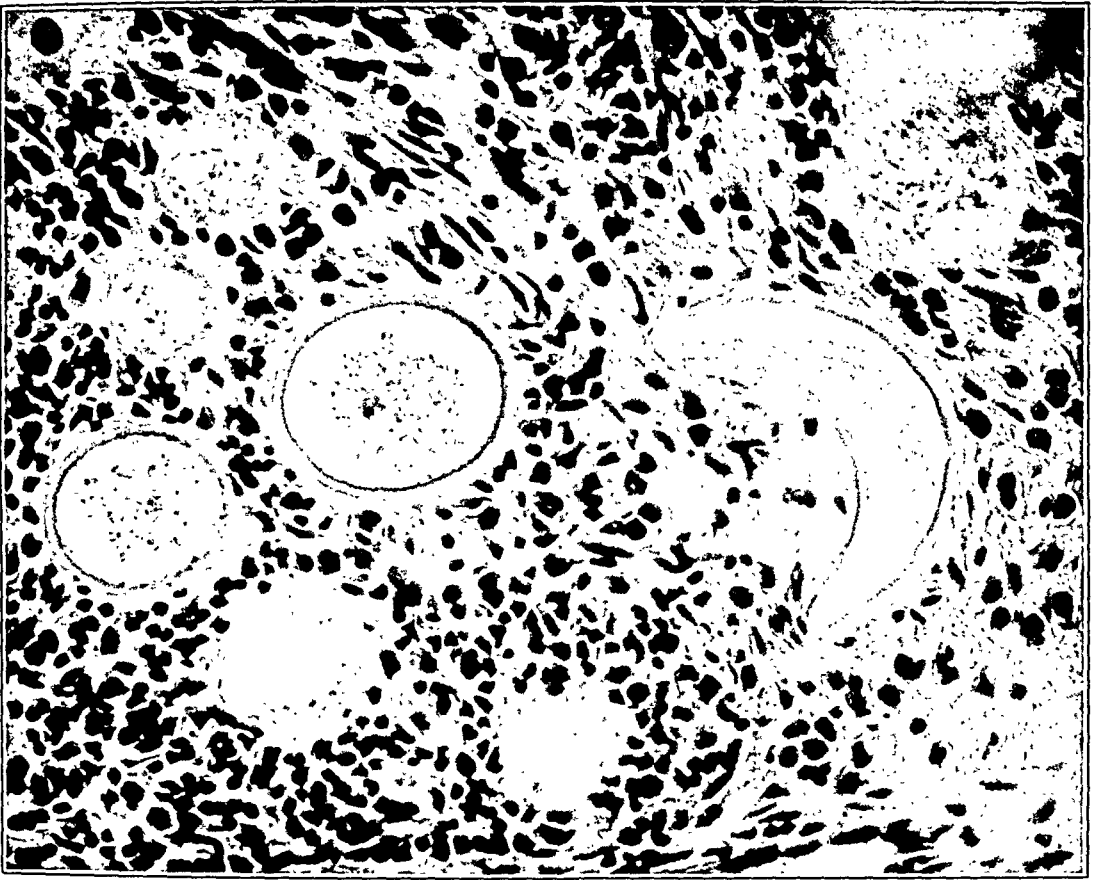
$$(c) \text{ Inclusion-nuclear index} = \frac{\text{Volume of inclusion}}{\text{Volume of nucleus}} \times 100.$$

The individual cell determinations made on the infected cells were analyzed by simple statistical measurements of variation; namely, averages, standard deviations and coefficients of variability with their probable errors.

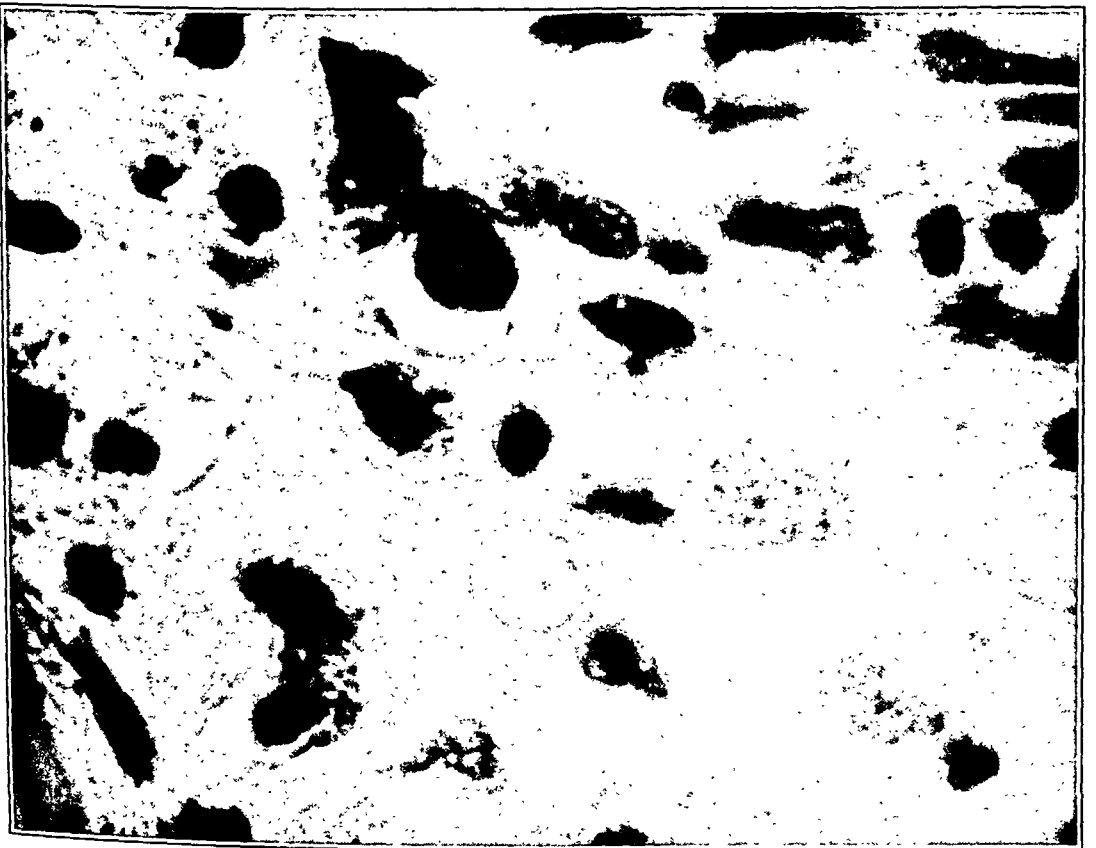
## RESULTS

*Cell Volume:* Care was taken to select normal secretory ducts which were of approximately the same size as those in which the inclusions are usually found. The average volume of 747 normal cells measured was found to be 613.2 cubic microns. It is interesting to note that the variation in the four sets of calculations made was of the order of ten. In order to determine the effect of inclusion-laden cells upon the adjacent seemingly normal ones the average volume of 597 unaffected cells in infected ducts was determined. These cells appeared to be, on casual inspection, somewhat compressed and small. But in spite of this appearance their average volume proved to be somewhat over 200 cubic microns greater than that of the normal duct cells. A few nuclear counts were made and it was found that there were in every instance fewer nuclei in such a duct as contrasted with the normal. Nuclear measurements also proved to be greater than in the normal duct. From these observations it is clear that duct cells near others which are infected, though they look normal, react in the usual way to stains and contain abundant mitochondria, are in reality modified volumetrically.

The infected cells were divided into two groups based upon staining reaction and upon the presence of certain cytoplasmic inclusions to



3

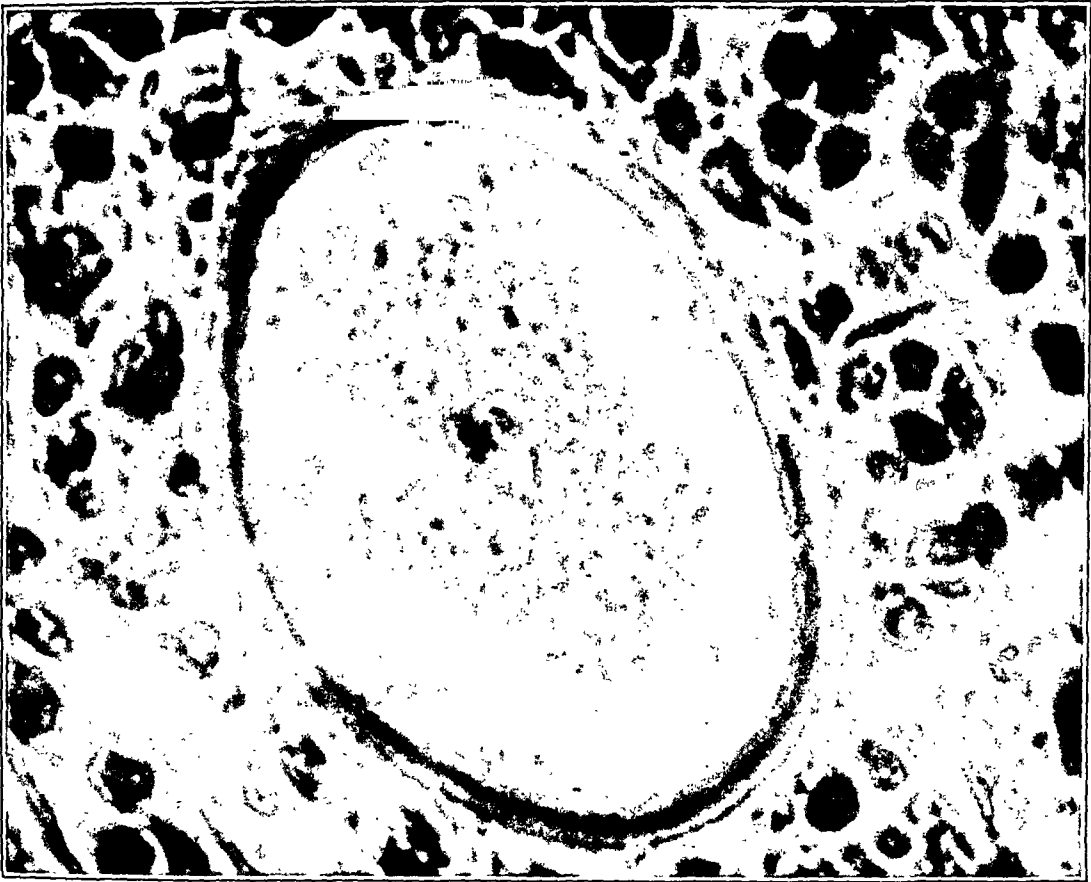


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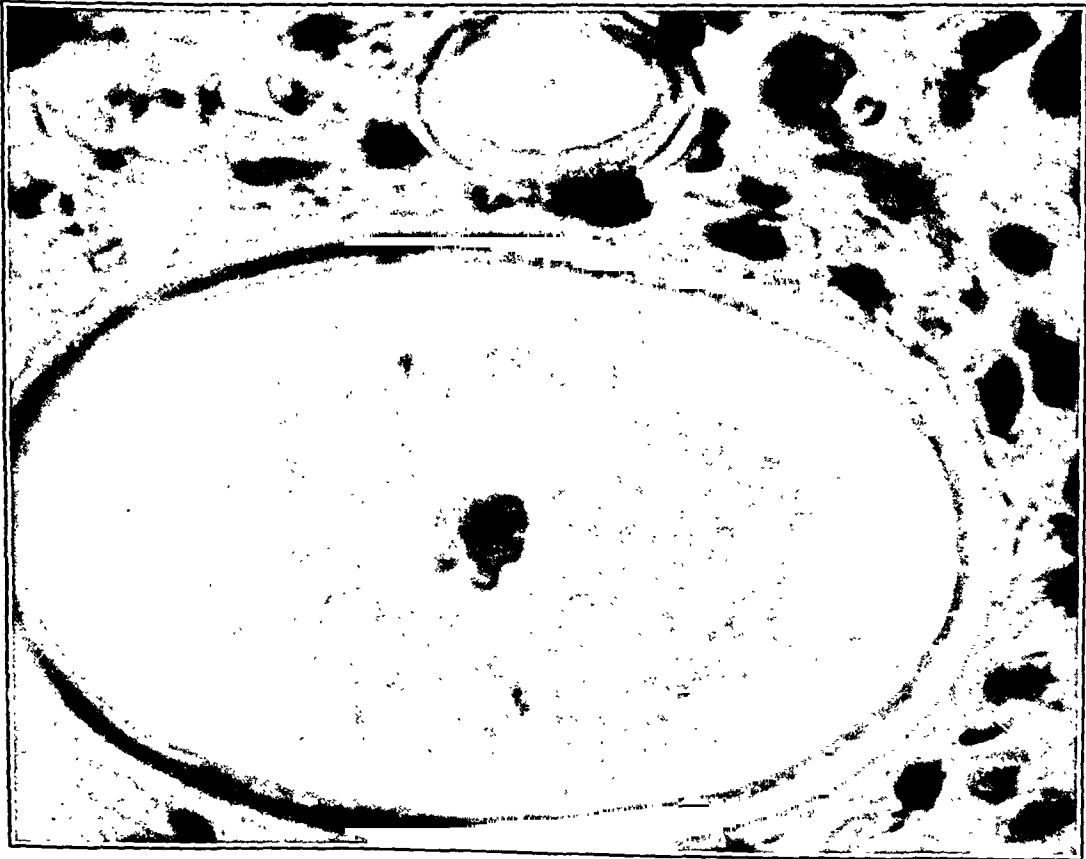
PLATE 133

FIG. 5. Trophic stage 58 microns in greater diameter. Beginning segregation of chromatin preparatory to first nuclear division. Abundant trophic material.  $\times 1500$ .

FIG. 6. Trophic stage 75 microns in its greater diameter. Early stage of first nuclear division.  $\times 1500$ .



5



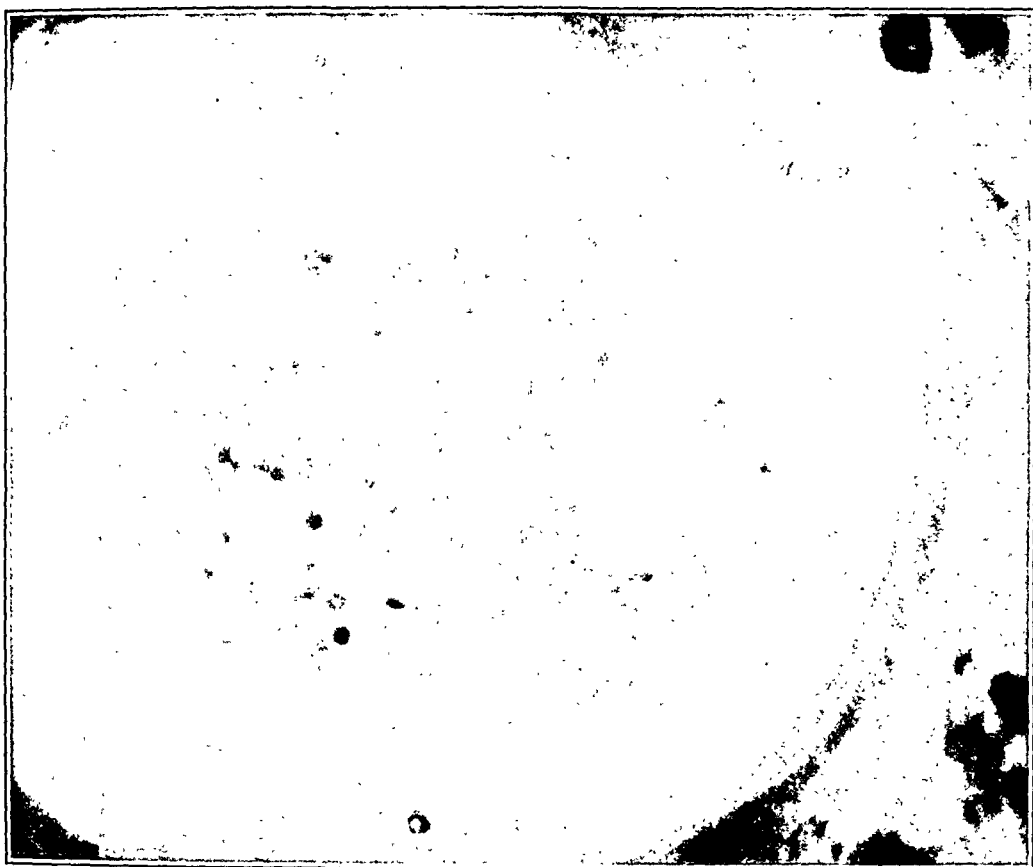
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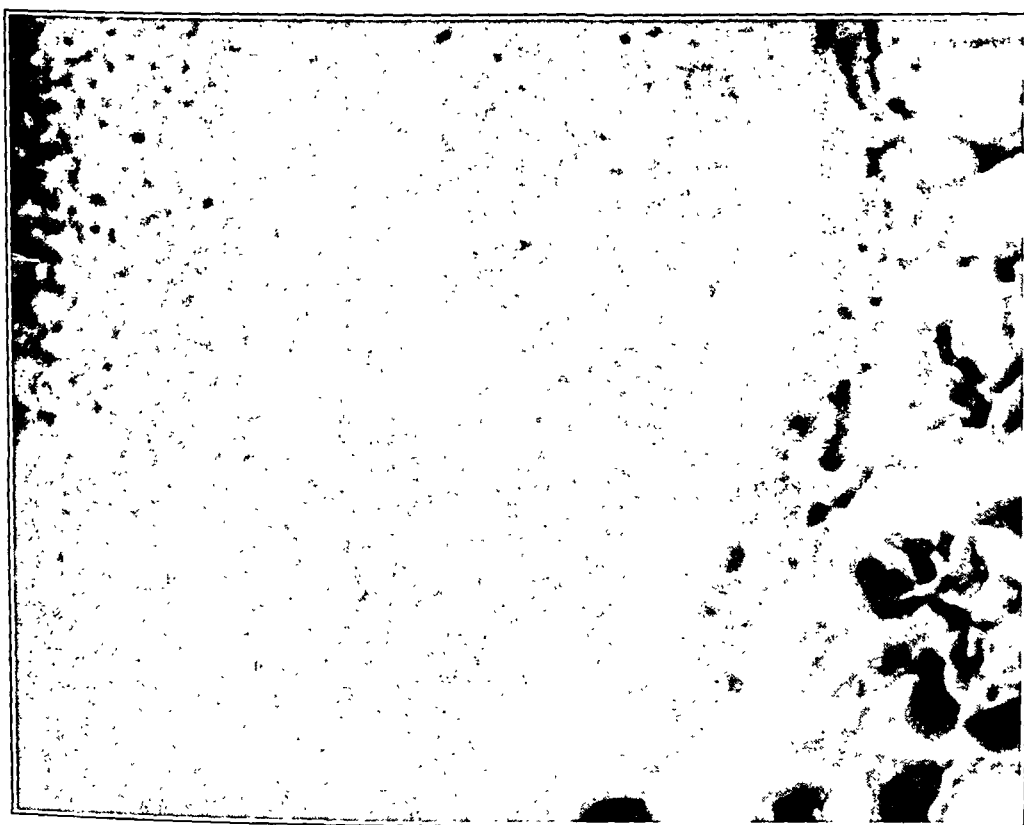
PLATE 134

FIG. 7. A portion of a parasite about 150 microns in diameter with thickened laminated wall and multiple nuclei. Twenty-eight nuclei were visible in the one section of this organism.  $\times 1500$ .

FIG. 8. A portion of a parasite 210 microns in diameter, closely packed with maturing spores. Cytoplasmic division has now taken place.  $\times 1500$ .



7

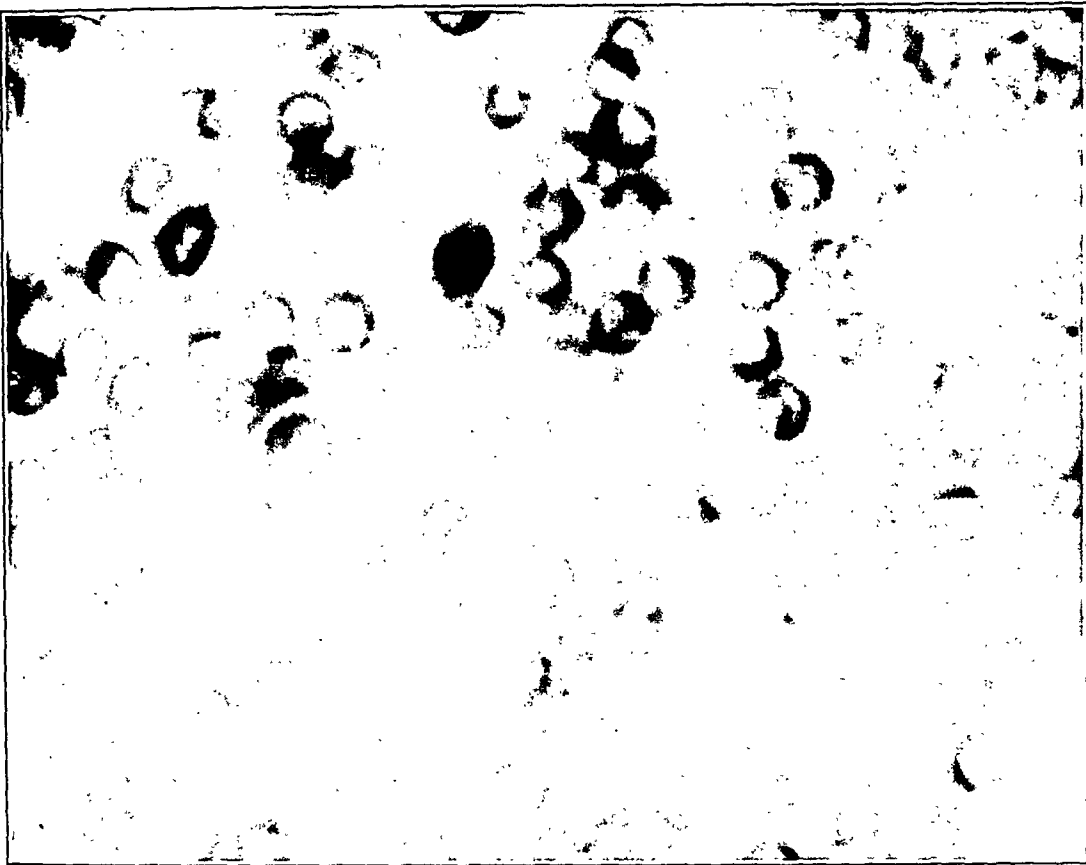


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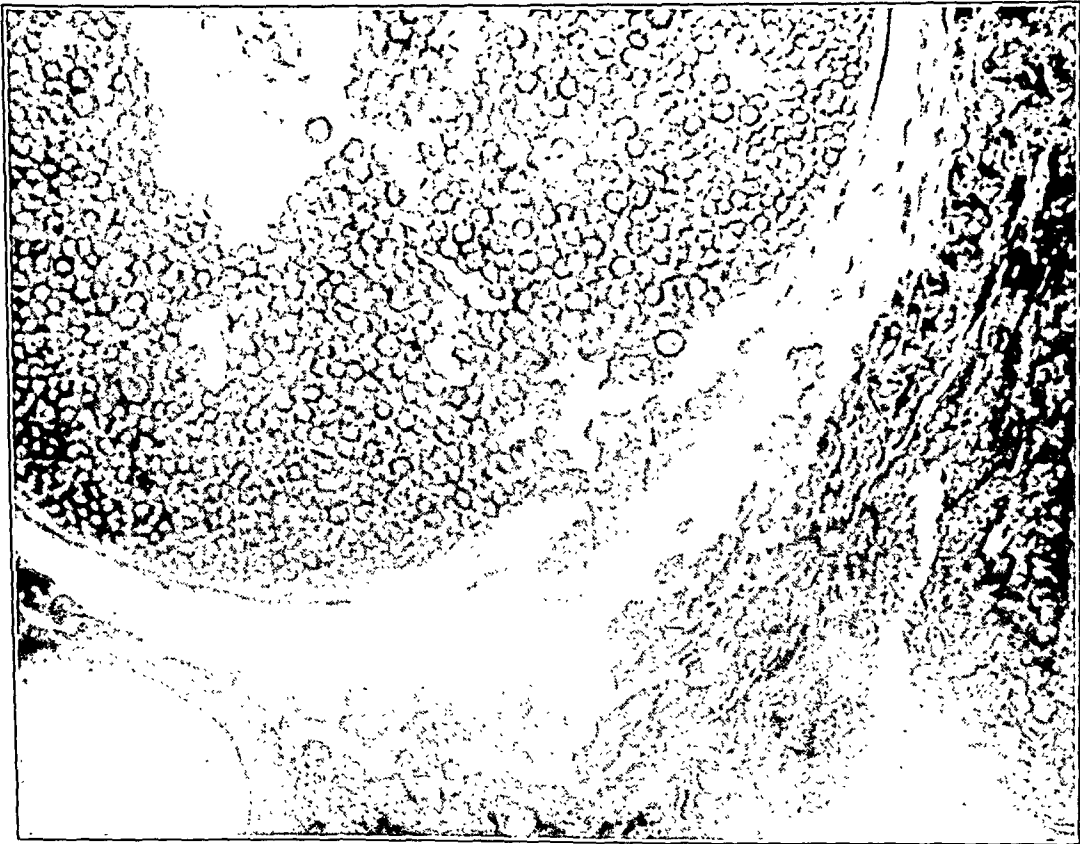
PLATE 135

FIG 9. Immature spores about 5 microns in diameter from the interior of a parasite in a somewhat later stage than in the preceding figure.  $\times 1500$ .

FIG. 10. The escape of ripe spores through the pore. The thickened marginal annulus is well shown. About  $\times 400$ .



9



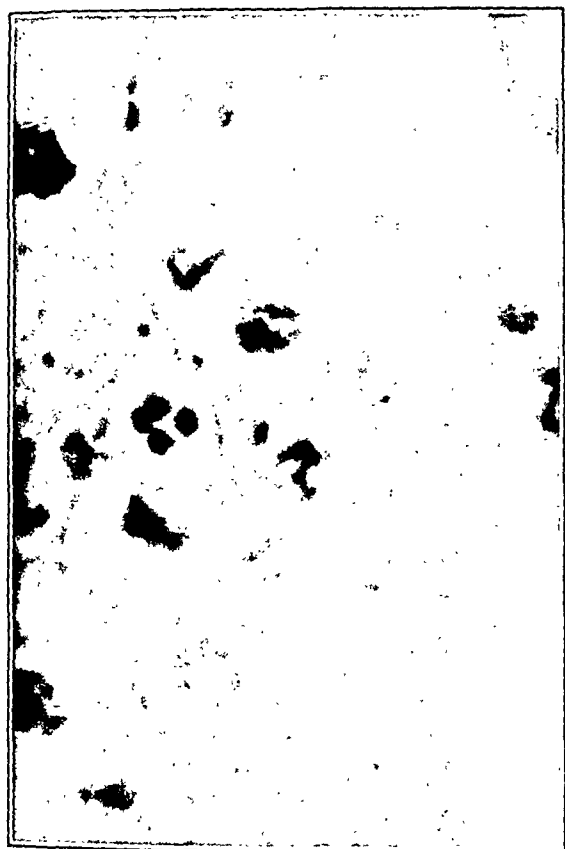
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PLATE 136

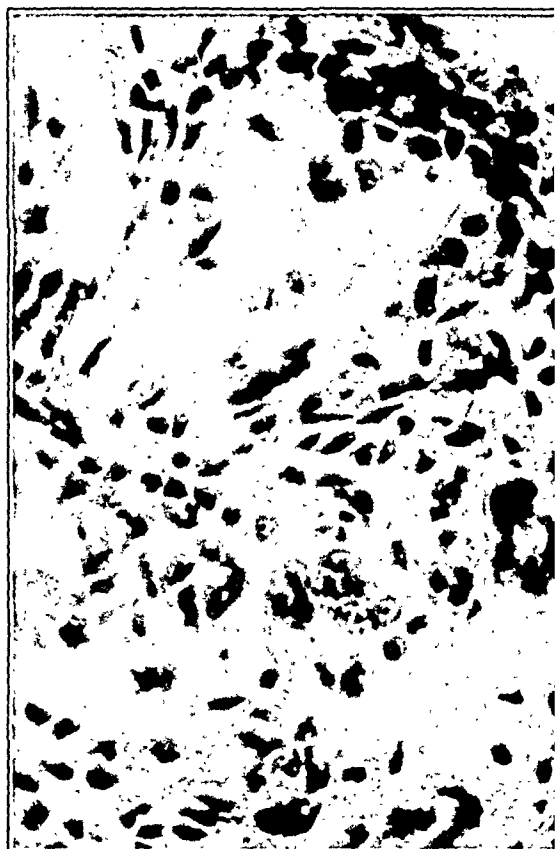
FIG. 11. Practically mature spores showing centrally grouped spherules with structureless material about them and well defined capsular membranes. About  $\times 2000$ .

FIG. 12. Foreign body giant cell reaction about old sporangium. Portions of wall and spores included within multinucleate foreign body giant cells.  $\times 300$ .

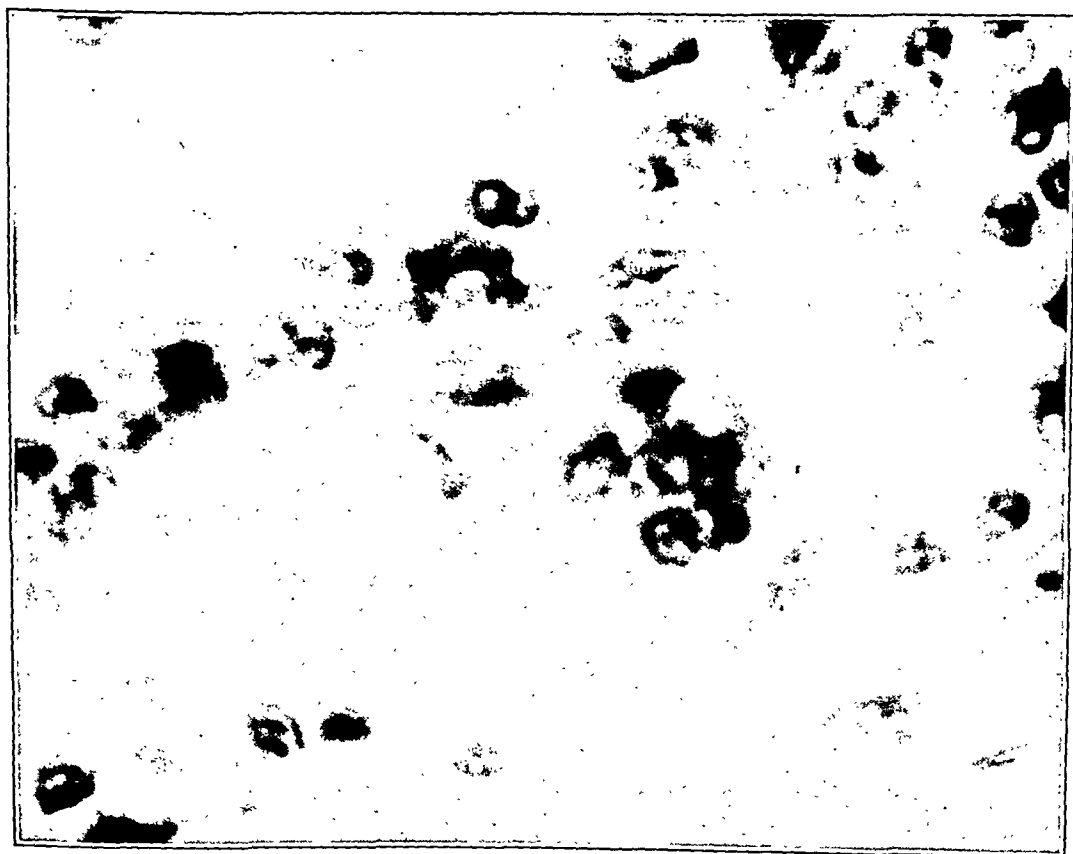
FIG. 13. Multinucleate foreign body giant cell containing many immature spores. Portion of wall of ruptured sporangium at bottom of field.  $\times 500$ .



11



12



13



be described in a forthcoming publication. In the first group, classed cells with "early inclusions," were placed those cells in which the cytoplasmic inclusions had not yet appeared and the small nuclear inclusions of which were acidophilic when stained by Giemsa's method. In the second group, designated cells with "late inclusions," the cytoplasm, in contrast contained many inclusions, and the nuclei exhibited large inclusions which were intensely basophilic in reaction when stained by the same method. Such marked characteristics admitted of no shading over from one class into the other in the arrangement of the data nor in the selection of the cells for measurement. Another useful point in classification was that nuclear hypertrophy was marked in the cells with "early inclusions" though the cytoplasm was but little altered. In the selection of material, however, little attention was paid to the cell size because doing so would obviously yield results which did not approximate the truth.

The volume of the cells containing "early inclusions" was not appreciably altered from that of the seemingly normal, but actually

TABLE I  
*Average Volumes of Cells, Nuclei and Their Inclusions*

	Cell volume in cubic microns	Nuclear vol- ume in cubic microns	Inclusion volume in cubic microns	Number of cells
Normal duct cells	613.2	149.1		747
Normal cells in infected ducts	816.0	160.1		597
Cells with early inclusion bodies	872.2	260.8	44.6	150
Cells with late inclusion bodies	2111.9	694.9	210.2	150

slightly enlarged, cells with which they were in intimate association. This group of 150 cells showed an average volume of 872.2 cubic microns. Despite this fact the nucleus had increased by approximately 100 cubic microns. The volume of the cells containing "late inclusions" was larger by more than 300 per cent than that of the normal duct cells, the mean value being 2111.9 cubic microns. The figures dealing with the changes in cellular volumes are given in detail in Table I.



stages of acute mural endocarditis. The pockets are formed secondarily, following the mechanical excavation of the thickened areas, by the regurgitating blood impulse after an insufficiency of the aortic valve has been established. Krasso<sup>11</sup> maintains that primarily all types of pockets are formed on the basis of endocardial thickenings which are caused mechanically by the force of the regurgitating blood stream, but infectious thrombi with organization, formed in such areas, might also play a rôle in the formation of circumscribed endocardial thickenings.

Schmincke<sup>12</sup> and Borst<sup>13</sup> are of the opinion that the formation of endocardial pockets is a sign of functional adaptation.

Sotti<sup>14</sup> holds that most of the pockets are abnormal muscle bridges converted into connective tissue, or aberrant muscles, or chordae tendineae, and therefore should be classified rather as malformations of the heart.

Ribbert,<sup>15</sup> in Henke and Lubarsch's handbook, says that several causes might lead to pocket formation; a primary circumscribed endocarditis, a continuous friction of thickened or calcified aortic leaflets of the mitral valve upon the opposite endocardial surface of the interventricular septum, the mechanical irritation of the regurgitating blood in aortic insufficiency, and finally, congenital anomalies.

The foregoing short survey of the literature shows the different views expressed as to the origin of endocardial pockets. While many writers believe they occur on a purely mechanical basis, others believe them to be of inflammatory origin, while the opinion is expressed by some authors that they are formed on the basis of congenital malformation. A possible relation of the endocardial pockets to the type of valvular disease has not attracted attention.

The present study was undertaken, first, to determine whether or not by the use of serial sections one definite etiological factor might be demonstrated as to the cause of the formation of pockets; secondly, to see if in different types of valvular diseases a different cause could be found to explain such formations. Endocardial pockets of the left auricle which were present in one case were similarly studied.

## METHODS

Six hearts were studied. A detailed description of the hearts will be given subsequently. The hearts were obtained from routine post-

## ENDOCARDIAL POCKETS \*

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### INTRODUCTION

In cases of insufficiency of the aortic valve, a coincidental and striking finding is that of endocardial pockets imitating the form of aortic valve leaflets on the surface of the interventricular septum of the left ventricle. These pockets are often multiple, their openings directed toward the aorta. Even more rarely such pockets are observed with their openings directed toward the apex of the heart.

### LITERATURE

Zahn,<sup>1</sup> who first directed attention to these formations in aortic insufficiency, interpreted them as anatomical signs of incompetence of the aortic valves. He believed that such pockets occurred on the basis of simple endocardial thickenings which were brought about by the chronic irritation of the impulse of the regurgitating blood. In his opinion, the prolonged irritation of the regurgitating blood produced the pockets or pseudovalves only secondarily. He observed plain endocardial thickening in the left auricle in cases of insufficiency of the mitral valve, but he did not describe pockets in the left auricle. Herxheimer,<sup>2</sup> Dewitzky,<sup>3</sup> Rosenbusch,<sup>4</sup> Wilke,<sup>5</sup> and Cohn,<sup>6</sup> similarly believe in the mechanical genesis of circumscribed endocardial thickenings and endocardial pockets. Kaewel,<sup>7</sup> who states that the formation of endocardial pockets may aid in the diagnosis of aortic insufficiency, also traces back their origin in most of the cases to the mechanical irritation of the regurgitating blood. He thinks that continuous pressure of a thickened aortic leaflet of the mitral valve upon the opposite side of the interventricular endocardium of the left ventricle often cannot be ruled out as a contributory cause. Ziegler,<sup>8</sup> Aschoff,<sup>9</sup> and later Böger<sup>10</sup> emphasize that circumscribed endocardial thickenings are primarily inflammatory in nature, end

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tracted and to some extent adherent to one another. A few small, recent vegetations were found attached to the free margins. The mitral leaflets were markedly thickened, in part calcified, and covered with large, partly firm and partly friable vegetations. Throughout the aortic leaflet of the mitral valve on its ventricular aspect, several ulcers were noticeable. The endocardial surface showed throughout the interventricular septum of the left ventricle, beginning from an area of about 3 cm. below the aortic valve and extending down to an area of about 2 cm. from the apical portion, a number of pockets. These pockets varied in size from 4 to 12 mm. in width. Some of the pockets extended in the form of fibrous bands over several trabeculae carneae and reached the papillary muscles. All pockets were open in the direction of the aortic valve. While some pockets were found in the vicinity of vegetations of the aortic leaflet of the mitral valve, others showed no apparent relation to these vegetations. There were no thrombi close to the pockets. The cusps of the pockets were grayish in color, firm in consistency, and free from vegetations.

*Histological Examination:* The histological findings in the pockets of both cases were about identical. The endocardium in some of the sections was fibrosed and showed only a moderate number of nuclei. Many sections, however, showed a diffuse infiltration of lymphocytes, endothelial cells, and a few polymorphonuclear leukocytes and eosinophiles. The cells were apparently more abundant close to the point of attachment of the cusp of the pockets to the endocardium. Some slides showed the extension of the inflammatory cells into the subendocardial layer. The heart muscle fibers found in the subendocardial region were markedly atrophic, their cytoplasm distinctly granular in appearance. There were only a few inflammatory cells present in these portions. The cusps of the pockets showed in some of the sections a dense fibrous tissue with only a few cellular elements, but some of the sections again showed large numbers of endothelial cells, lymphocytes and a few polymorphonuclear leukocytes. A few fields showed the formation of small-sized blood vessels extending from the endocardium into the cusps. There were remnants of blood pigment present. In some of the sections remnants of organizing thrombi were recognizable. Sections which were taken from the bands extending over the trabeculae carneae showed the latter to be surrounded by connective tissue and elastic fibers with

mortem examinations. No museum specimens were used for this investigation. The endocardial pockets with the adjacent myocardium were cut out, hardened in 10 per cent formalin and embedded in paraffin. Serial sections were made from the various pockets. The first, fourth, seventh, etc., sections were stained with iron hematoxylin and eosin; the second, fifth, eighth, etc., according to Van Gieson's method, while the third, sixth, ninth, etc., were prepared with a combination of iron hematoxylin and orcein stain, using a method described elsewhere (Saphir<sup>16</sup>).

### CASE REPORTS

#### *Endocardial Pockets in Hearts Showing Acute Ulcerative and Chronic Endocarditis (Subacute Bacterial Endocarditis with Positive Blood Culture of Streptococcus Viridans)*

CASE 1. *Pathological Diagnoses:* Acute ulcerative and vegetative endocarditis superimposed on chronic endocarditis of the aortic and mitral valves, with insufficiency of both valves.

*Gross Description:* The heart was enlarged, weighing 450 gm. The free margins of the cusps of the aortic valve showed several fresh vegetations. The cusps themselves were retracted and shortened. There were a few small ulcers throughout the ventricular surface of the left aortic cusp. Many recent vegetations were found extending over the aortic leaflet of the mitral valve. The free margins of the mitral valve leaflets were retracted and thickened, the chordae tendineae were firm and shortened. The endocardium below the left aortic cusp showed many recent, grayish red vegetations. The endocardium surrounding them was of a grayish white color. At a distance of 6 mm. from these vegetations, in an area 2 cm. below the aortic valve, situated on the interventricular surface of the left ventricle there were two small circumscribed endocardial pockets measuring 3 and 5 mm. in width. These pockets were open toward the aortic valve.

CASE 2. *Pathological Diagnoses:* Acute vegetative, superimposed on chronic endocarditis of the aortic valve. Acute vegetative and ulcerative endocarditis of the mitral valve superimposed on chronic endocarditis. Insufficiency of the aortic and mitral valves.

*Gross Description:* The heart was enlarged, weighing 500 gm. The free margins of the aortic cusps were thickened, the cusps re-

mitral valve leaflets showed no changes. The conus arteriosus sinister (Krasso) was apparently the seat of a relative stenosis. The endocardium of the left ventricular surface of the interventricular septum showed many thickened areas which were of stringy appearance and which were found in an area about 2 cm. below the aortic valve. In and below this region several typical pockets were found which measured from 2 to 8 mm. in width. The cusps of these pockets were firm in consistency. Most of the pockets were in one horizontal row, but several were found above and below this row. All these pockets were open toward the aortic orifice. Just below the left aortic cusp there was one pocket which measured 5 mm. in width, and which was open toward the apex. The endocardium close to this pocket was grayish white in color and of much firmer consistency than the surrounding portions.

*Histological Examination:* The endocardium in the region of the pockets was fibrosed. There was an occasional lymphocyte and endothelial cell found, but the tissue in general was very poor in cells. In some portions the endocardium was hyalinized. The fibrosis extended into the surrounding subendocardial layer. But here, too, very few cellular elements were seen. The muscle fibers close to the endocardium were atrophic and in some fields present in the form of a light pink-stained material assuming the shape of heart muscle fibers. By use of the Van Gieson method these fibers stained yellow. The cusps of the pockets showed a dense connective tissue overgrowth with a varying number of fibroblasts. There were hardly any blood vessels found in this portion nor was there any evidence of organization. Both types of pockets, the ones with the openings directed toward the apex and the ones with the mouths open toward the aorta, showed identical lesions. The elastic tissue stain showed an abundance of elastic fibers throughout the cusps. Some of the sections showed clearly the extension of the elastic lamellae from the endocardium into the cusps.

#### *Endocardial Pockets in Hearts Showing Rheumatic Endocarditis*

CASE 5. *Pathological Diagnoses:* Healed endocarditis of the aortic valve. Stenosis of the aortic orifice.

*Gross Description:* The heart was enlarged and dilated, weighing 400 gm. The free margins of the aortic valve were thickened and

many lymphocytes and endothelial cells. The trabeculae carneae themselves were outlined indistinctly and many of them could be recognized only with the aid of the Van Gieson stain. Between the fibers, inflammatory cells were observed. The elastic stain showed an abundance of elastic lamellae of the cusps. They were found in parallel rows. Some of the fields showed the extension of the endocardial elastic lamellae into the cusps.

*Endocardial Pockets in Hearts Showing Syphilitic Involvement  
of the Aortic Valve*

CASE 3. *Pathological Diagnoses:* Syphilitic aortitis with involvement of the aortic valve. Insufficiency of the aortic valve.

*Gross Description:* The heart was hypertrophic and dilated, weighing 650 gm. The aorta showed characteristic lesions of syphilitic aortitis. The cusps of the aortic valve showed a separation of the commissures extending over areas measuring 3 to 4 mm. in extent. There was a marked insufficiency of the aortic valve. The aortic leaflet of the mitral valve was free from pathological changes. The upper portion of the left ventricle, just below the aortic valve, seemed much narrowed as compared with the size of the remainder of the ventricle which was markedly dilated and hypertrophic. The impression was gained that this area which Krasso calls "conus arteriosus sinister," was the seat of a relative bottle-neck stenosis. The endocardium of the interventricular septum of the left ventricle showed a series of pockets in four parallel horizontal rows. The pockets measured 2 to 6 mm. in width. In the region of the trabeculae carneae a few connective tissue bridges were seen. All these pockets were open toward the aorta. Just below the left aortic cusp, however, another pocket was found which measured 3 mm. in width. This pocket was open toward the apex of the heart. In the vicinity of this pocket, the endocardium was grayish white in color and thickened.

CASE 4. *Pathological Diagnoses:* Syphilitic aortitis with involvement of the aortic valve. Insufficiency of the aortic valve.

*Gross Description:* The heart weighed 700 gm. It was markedly hypertrophic and dilated. The aortic lesions were characteristic of syphilitic aortitis. There was a distinct separation of the commissures of all three cusps and insufficiency of the aortic valve. The

remnants of blood pigment and phagocytic cells. The elastic tissue stain showed a great number of elastic fibers without any particular arrangement. Some of the sections showed these fibers extending from the endocardium into the cusps.

## DISCUSSION

### *Endocardial Pockets in Hearts Showing Subacute Bacterial Endocarditis*

The endocardial pockets which were found in the two cases of subacute bacterial endocarditis showed evidence of inflammation. There were still remnants of inflammatory cells, mainly lymphocytes, a few eosinophiles, endothelial cells and occasional polymorphonuclear leukocytes. Besides, a new formation of small-sized blood vessels was easily noticeable. Young connective tissue fibers were seen throughout some of the sections, while other sections showed scar tissue which in some portions was hyalinized. The sections emphasized the importance of serial sections as the only means of studying these changes. It easily can be understood why, by the use of only a few sections, hyalinized scar tissue alone might have been found. The sections showed that there must have been primarily an acute inflammatory exudate which secondarily became organized. Whether this was primarily a mural endocarditis or whether the initial lesion was brought about by contact with the diseased aortic leaflet of the mitral valve cannot be decided.

Both of our cases showed an insufficiency of the aortic valve. It seems plausible that the force of the regurgitating blood and pressure during diastole, continuously irritating the primary inflammatory area of the parietal endocardium, finally resulted in the formation of the pockets. The question arises whether the pockets were formed during the process of organization of the circumscribed parietal endocarditis, or whether they were formed secondarily after the scar formation had been completed. It is conceivable that as soon as the insufficiency of the valve was established, and as soon as irregularities were formed along the course of the regurgitating blood and pressure, the irregularities provided a foothold for the regurgitating blood which, with oft repeated insults to these areas, finally led to the formation of pockets. These irregularities might be either an organizing exudate or circumscribed endocardial thickenings, *i. e.*,

showed adhesions between the lateral portions of the cusps, producing a stenosis of the aortic orifice. The myocardium histologically showed several Aschoff bodies. The endocardium in an area about 1 cm. below the left aortic cusp showed a pocket measuring 8 mm. in width. This pocket was open toward the apex. The margin of this pocket was thin and sharp. The endocardium in the neighborhood of this pocket was thickened and fibrosed.

*Histological Examination:* The sections of the endocardium showed a marked increase of connective tissue with only very few nuclear elements. The fibrosis extended into the surrounding portions of the myocardium. The heart muscle fibers in this region were apparently atrophic. The cusps themselves showed a hyalinized connective tissue with few spindle-shaped cells. There were no blood vessels found, nor was there any other evidence of organization.

CASE 6. *Pathological Diagnoses:* Acute verrucous, superimposed on chronic endocarditis of the aortic and mitral valves. Insufficiency of both valves.

*Gross Description:* The heart was enlarged and dilated weighing 300 gm., (patient was a child 6 years of age). The cusps of the aortic valve were shortened and retracted. Their free margins were studded with a row of bead-like vegetations. The free margins of the mitral valve were thickened and retracted. Some of the chordae tendineae were fused by confluence. They were much shorter than normal, and thickened. The myocardium upon histological examination showed many Aschoff bodies. In an area about 2 cm. above the mitral valve, the auricular endocardium showed two pockets. The pockets measured 3 and 5 mm. in width. They were quite separate and were open toward the mitral valve. The surrounding portions of the endocardium were grayish white in color and thickened.

*Histological Examination:* The histological examination of the endocardium in the region of the pockets showed a diffuse infiltration of many polymorphonuclear leukocytes, a few lymphocytes and endothelial cells. There was a moderate amount of connective tissue with many spindle-shaped cells. The cusps similarly contained a large number of polymorphonuclear leukocytes, many lymphocytes, endothelial cells, and, in addition, many connective tissue fibers with a large number of fibroblasts. There were small-sized blood vessels found extending into the cusps. Some of the fields showed



This location of such pockets was commonly encountered by most of the investigators. Histologically none of the pockets showed remnants of an acute inflammatory exudate. In both instances they revealed only connective tissue which was poor in nuclei. Ribbert believes that the thickened endocardium in such areas was due to an extension of the syphilitic process from the valves to the endocardium. But these areas showed histologically nothing characteristic of syphilis. Libman,<sup>17</sup> in discussing Cohn's paper, does not believe in the syphilitic origin of endocardial pockets. Krasso believes that primary endocardial thickening, similar to that preceding the formation of pockets open toward the aorta, is due to the continuous pressure of the regurgitating blood in aortic insufficiency. He states that in the case of a relative stenosis of the conus arteriosus sinister, such thickened areas were secondarily transformed into pockets by the force of the systolic pressure. The location of such pockets just below the aortic valve, however, makes it seem unlikely that they were primarily the result of mechanical pressure of the regurgitating blood. Furthermore, there are cases reported, without evidence of insufficiency of the aortic valves, showing such pockets. Case 5 of this series, similarly did not disclose an aortic regurgitation.

The following table is offered to show the reported cases in which mention was found of pockets open toward the aorta. The table gives the name of the author, the diagnosis of valvular disease, the number of the particular case, and the number of pockets.

end stages of a parietal endocarditis. In many sections of the cusps of the pockets, blood vessels were seen extending from the endocardium through the bases of the pockets into the cusps. In addition, the cusps also showed inflammatory cells and blood pigment. These findings speak more for the fact that the formation of the pockets occurred during the period of organization. If the pockets had been formed secondarily upon an endocardial scar after the inflammation had subsided, we would rather expect to find in the cusps of the pockets hyalinized connective tissue without blood vessels or remnants of inflammatory exudate, mere evidence of mechanical irritation. I believe, therefore, that the regurgitating blood and pressure in aortic insufficiency produced the pocket formation in the area which was the seat of a parietal endocarditis undergoing organization. The finding of remnants of muscle fibers, with marked atrophy but without signs of inflammation in some portions, showed that the atrophy was more likely the result of a continuous mechanical pressure which brought about the formation of pockets, than evidence of past inflammations extending into the myocardium.

*Endocardial Pockets in Hearts Showing Syphilitic Involvement  
of the Aortic Valve*

Both of our cases showed pockets which were open in the direction of the aorta. But in addition, each case showed one pocket which was open in the direction of the apex of the heart. The sections of the pockets open toward the aorta showed an abundance of connective tissue with hardly any nuclear elements, no blood vessels, but many elastic fibers. A primary inflammation and secondary organization therefore can be ruled out. The only explanation I can offer for the formation of these pockets lies in the aortic regurgitation. The degree of insufficiency of the aortic valve in both cases was very marked. It must be assumed that the regurgitating blood and pressure acting as a chronic irritant primarily produced circumscribed fibrosed areas of the endocardium. As soon as irregularities of the endocardium were formed, the continuous regurgitation with formation of eddies in these regions finally resulted in the formation of pockets.

The pockets which were open toward the apex were found below the left aortic cusp very close to white, fibrosed areas in both cases.

*Nuclear Volume:* The volume of the nucleus of the normal duct cell, when estimated by the procedure employed, was found to be 149.1 cubic microns. The mean diameter of the nucleus was calculated to be 6.76 microns. The observed diameter of the nucleus averaged 6.55 microns when a control series of 450 nuclei were measured. The mean nuclear volume of the apparently unaltered cells in the infected ducts was computed to be 160.1 cubic microns. This rise in nuclear volume however is so slight that it would be unwise to attach much significance to it.

Nuclear hypertrophy in the cells with "early inclusions" increased the volume of the nucleus to 260.8 cubic microns, but the cytoplasmic enlargement did not keep pace with the nuclear change. The duct cells with "late inclusions" showed an even greater increase in volume for at this stage the average nuclear size reached 694.9 cubic microns (Table I).

*Inclusion Volume:* The inclusions, found in the cells with beginning infections, when measured showed an average volume of 44.6 cubic microns. The mature inclusions, on the other hand, had increased in size so that they were more than 300 per cent greater in volume than those of the cells with early infections. The volume of the inclusions in these cells was 210.2 cubic microns or almost as large as the entire nucleus in cells with "early inclusions."

*Nuclear Cell Index:* This expression of the nucleus cell relation in terms of volume was obtained by dividing the volume of the nucleus by the volume of the cell and multiplying the result by 100 so that it could be expressed in percentages. The nuclear cell index for the normal cells was 24.27, which means that the nucleus formed practically one-fourth of the cell volume. The normal cells in the infected ducts showed a definite and significant change in this relation because the nuclear cell index dropped to 20.44. In this instance the nucleus formed one-fifth of the total cell volume. No microscopically visible changes were observed capable of explaining why this alteration in cell and nuclear volume should occur.

Since the infected cells were measured individually it was possible to apply certain mensurations of variability which confirmed the propriety of separating them into two classes. It also provided a means of checking the accuracy of the method applied to their volumetric determinations.

The mean nuclear cell index in the cells with "early inclusion

TABLE I

*Case Reported with Pockets open Toward the Aorta*

Author	Main diagnosis	Case No.	Number of systolic pockets
Wilke	Stenosis of the aortic orifice due to a papillary tumor of the aortic valve.	3	Several
Wilke	Recurrent endocarditis of the aortic valve. Stenosis of the aortic orifice and insufficiency (?) of the aortic valve.	4	Several
Kaewel	Syphilitic involvement of the aortic valve. Insufficiency of the aortic valve.	6	2
Kaewel	Syphilitic involvement of the aortic valve. Insufficiency of the aortic valve.	7	1
Kaewel	Syphilitic involvement of the aortic valve. Insufficiency of the aortic valve.	8	2
Kaewel	Acute, superimposed on chronic endocarditis of the mitral and aortic valves.	21	3
Böger	Healed thrombo-endocarditis, ulcerosa lenta. Stenosis of the aortic orifice and insufficiency of the aortic valve.	7	2
Böger	Healed rheumatic endocarditis of mitral valve.	12	1
Krasso (first paper)	Recurrent malignant endocarditis. Insufficiency of the aortic valve and moderate stenosis of the aortic orifice.	1	1
Krasso (second paper)	Healed endocarditis of the aortic and mitral valves. Insufficiency of the aortic valve and stenosis of aortic orifice.	1	1
Krasso (second paper)	Recurrent malignant endocarditis. Insufficiency of aortic valve and stenosis of aortic orifice.	2	1
Krasso (second paper)	Recurrent verrucous endocarditis. Insufficiency of aortic valve and stenosis of aortic orifice.	3	1
Krasso (second paper)	Syphilitic involvement of the aortic valve. Insufficiency of the aortic valve.	5	2
Krasso (second paper)	Healed endocarditis of aortic valve. Insufficiency of aortic valve.	6	1
Krasso (second paper)	Healed endocarditis of aortic valve. Syphilitic involvement of the aortic valve. Insufficiency of aortic valve.	8	Several

Seven cases out of the fifteen shown in the table were the seat of an unquestionable stenosis of the aortic orifice. Five cases showed a syphilitic involvement of the aortic valve; but the hearts of these cases were the seat of a marked hypertrophy and dilatation leading, as Krasso specifically pointed out, to a relative stenosis of the aortic conus. In his case (No. 6) showing an insufficiency of the aortic valve without organic stenosis of the orifice, Krasso emphasized the presence of a relative stenosis of the aortic conus. In Kaewel's case (No. 21) showing an acute, superimposed on chronic endocarditis of the aortic and mitral valves, the diagnosis of stenosis of the aortic orifice was not mentioned, but the hypertrophy and dilatation of the heart was emphasized. Böger's case (No. 12) showed a healed rheumatic mitral endocarditis. But neither the size nor the weight of the heart was given, so that nothing can be said about a possible relative stenosis of the aortic conus. Both of our cases of syphilitic involvement of the aortic valve, in which pockets open toward the apex were found, were the seat of a relative stenosis of the aortic conus brought about by the marked hypertrophy and dilatation of the heart. It is possible that the friction of the systolic blood stream, and pressure, is sufficient to produce a mechanical irritation of an area situated in the region of the stenosed conus. At the same time, the continuous impulse of the systolic blood stream, and pressure, might result in the formation of pockets. Krasso calls pockets which are open toward the aorta diastolic pockets, and those open toward the apex, systolic pockets. It seems that this nomenclature is justifiable and should be adopted.

### *Endocardial Pockets in Rheumatic Endocarditis*

Case 5, which showed a healed rheumatic endocarditis resulting in a stenosis of the aortic orifice, presented only one systolic pocket just below the aortic valve area. However, the surrounding endocardium was diffusely thickened. The sections of both, the cusp of the pocket and the surrounding endocardium, revealed no indications of organization or remnants of an inflammatory exudate. The sections showed only fibrous tissue with a few nuclear elements. The aortic leaflet of the mitral valve showed no changes. This pocket, similar to the systolic pockets of the last two cases, was apparently the result of the continuous irritation and friction of the systolic

blood stream and pressure upon the area below the stenosed aortic orifice, producing, first, simple thickenings of the endocardium with secondary formation of pockets. The pathogenesis of the systolic pockets of this case and of the last two cases is apparently identical.

Sections of the auricular pockets of the second case of this group showed the presence of inflammatory cells, lymphocytes, polymorphonuclear leukocytes, endothelial cells and a new formation of connective tissue. The surrounding portions of the myocardium showed similar inflammatory cells. It is evident that the primary changes were inflammatory in nature. As in the first group of cases, the impulse of the regurgitating blood directed upon an area of organizing parietal endocarditis of the left auricle, caused the formation of pockets after the insufficiency of the mitral valve was established.

This case is especially noteworthy because a search through the literature disclosed only one other case, described by Abbott,<sup>18</sup> which showed pockets in the left auricle. This author found a thick-walled endocardial pocket in the left auricle of a heart which was the seat of a large open foramen ovale and button-hole stenosis of the mitral orifice. The depths of this pocket lay in close contiguity to a muscular channel running from an accessory chamber in the right auricle. The auricular endocardium was greatly thickened. The histological details, however, are lacking and it is, therefore, difficult to decide whether this pocket was inflammatory in origin or evidence of another malformation of the heart.

The various pockets in our cases, therefore, seem of different origin. Some are primarily inflammatory in nature, results of organizing parietal endocarditis, while others seem to be the result of primary mechanical irritation. The formation of the pockets themselves, however, is in the final analysis, always caused by the force of either systolic or diastolic regurgitating blood columns and pressure.

In the discussion of the pathogenesis of diastolic endocardial pockets, the ultimate cause for their formation appears to be the regurgitation of blood and pressure. Wiggers<sup>19</sup> in his "Circulation in Health and Disease" states that in aortic insufficiency only a small volume of blood actually regurgitates, and that the essential dynamic disturbance is brought about not by the volume of blood which regurgitates, but by the regurgitation of pressure during diastole. However, more recently, Wiggers and Green<sup>20</sup> found that in

artificially produced aortic insufficiency, the total regurgitation under optimum conditions can equal 50 to 60 per cent of the normal tidal volume in the perfused heart. It seems unlikely that the regurgitating pressure in aortic insufficiency is able to act upon one circumscribed area of the endocardium and produce there, in time, diastolic pockets. It is more likely that the regurgitating pressure occurring without fluid movement would extend equally in the various directions. The only possible explanation of the pathogenesis of the formation of endocardial pockets lies in the assumption of a regurgitation of blood. The regurgitation of pressure might play an additional, but much less important rôle. Whether the actual friction of the regurgitating blood, or whether eddies formed by the regurgitating blood and directed upon a circumscribed portion of endocardium produce the chronic inflammation which is the basis for the formation of some of the pockets, cannot be decided.

All the sections revealed the presence of a great number of elastic lamellae. Some of the sections showed the direct continuation of the internal elastic lamellae of the endocardium into the pockets.

Wilke, as stated before, is of the opinion that the pockets are manifestations of functional adaptation. A similar view was more lately expressed by Borst. Functional adaptation, however, implies that the part involved adapts itself to new functional demands (Karsner<sup>21</sup>) and actually fulfills the demanded functions (Borst). The endocardial pockets, however, only resemble pockets of aortic valves. Even though they are brought about by the force of the blood stream and are often found to be multiple, yet they cannot have any marked function because they are small and hold only a very insignificant amount of blood. To fulfill a function, it would be necessary that the pockets be close enough together to allow their cusps to touch during diastole as aortic cusps do. If such pockets were found in an entire row just below the aortic valve, a teleologist might be justified in assuming them to be evidence of functional adaptation.

#### SUMMARY AND CONCLUSIONS

1. In two cases of subacute bacterial endocarditis of the aortic and mitral valves with insufficiency of the aortic valve, endocardial pockets with openings toward the aorta were found on the interventricular septum of the left ventricle. The initial lesion which

brought about the pocket formation was a circumscribed parietal endocarditis. The continuous regurgitation formed the pockets secondarily.

2. In one case of rheumatic endocarditis of the mitral valve with insufficiency of this valve, endocardial pockets were present in the left auricle. These pockets were open toward the mitral valve. They also were primarily inflammatory in origin and formed secondarily by the regurgitation after the insufficiency of the mitral valve had been established.

3. In two cases of syphilitic involvement of the aortic valve with insufficiency of this valve, endocardial pockets open toward the aorta were found. These pockets were caused primarily by the mechanical irritation of the regurgitating blood columns.

4. Two cases of syphilitic involvement of the aortic valve with insufficiency of this valve and marked stenosis of the conus arteriosus sinister, and one case of rheumatic endocarditis of the aortic valve with stenosis of its orifice, showed endocardial pockets on the interventricular surface of the left ventricle. These pockets were open toward the apex of the heart. They were brought about by the mechanical irritation of the systolic blood stream acting as a trauma upon the endocardium in the region of the stenosed portions.

5. Diastolic endocardial pockets are evidence in favor of the view of actual regurgitation of blood volume.

6. The nomenclature of "diastolic pockets" referring to those open toward the aorta and "systolic pockets" referring to those open toward the apex (Krasso) is justified.

7. Endocardial pockets cannot be regarded as manifestations of functional adaptation.

I am indebted to Prof. H. T. Karsner for his valuable suggestions.

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## DESCRIPTION OF PLATES

### PLATE 137

FIG. 1. Heart of Case 2. Diastolic pockets of the interventricular septum of the left ventricle.

FIG. 2. Heart of Case 3. Diastolic pockets on the interventricular septum and one systolic pocket below the left aortic cusp.

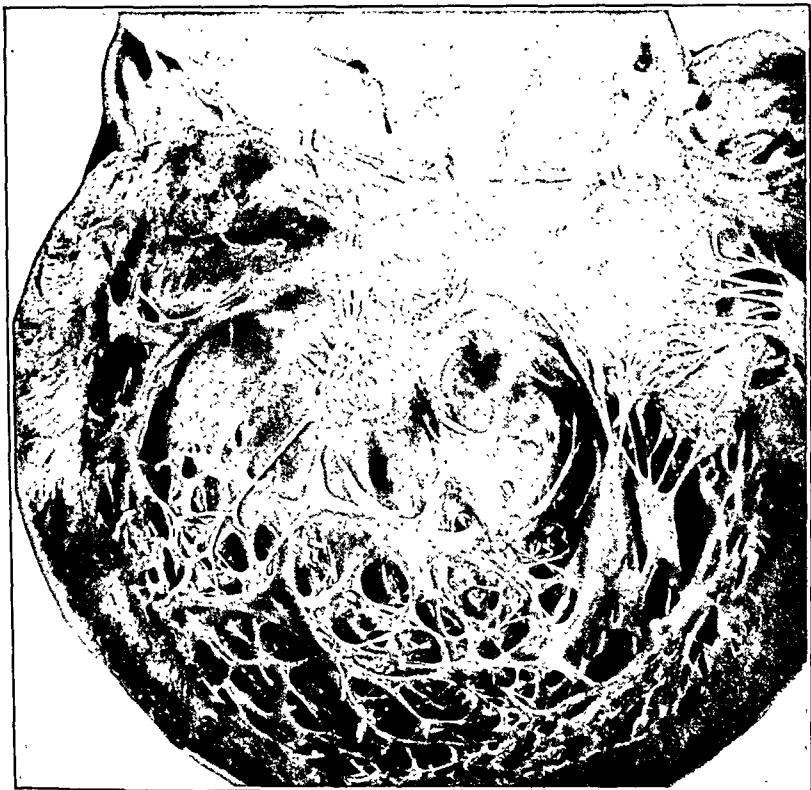
FIG. 3. Heart of Case 4. Diastolic pockets and one systolic pocket below the left aortic cusp. Note the marked dilatation of the heart.



I



2



3

PLATE 138

FIG. 4. Heart of Case 5. One systolic pocket.

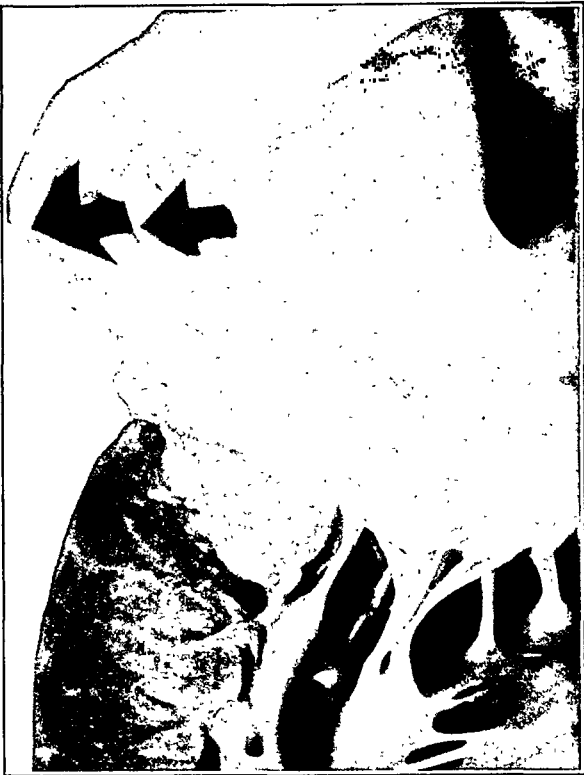
FIG. 5. Heart of Case 6. Note two endocardial pockets on the left auricular endocardium.

FIG. 6. Cusp of pocket of Case 2. Note the inflammatory cells. Iron hematoxylin and eosin preparation.  $\times 260$ .

FIG. 7. Section of base of pocket of Case 2. Note the inflammatory cells. Iron hematoxylin and eosin preparation.  $\times 260$ .



4



5



6



7

PLATE 139

FIG. 8. Cusp of pocket of Case 2. Note the newly formed blood vessels. Iron hematoxylin and eosin preparation.  $\times 180$ .

FIG. 9. Fibrous band extending over one of the trabeculae carneae. Note the abundance of elastic lamellae. Orcein and iron hematoxylin preparation.  $\times 80$ .

FIG. 10. Cusp of pocket and adjacent myocardium of Case 3. Note the spindle-shaped cells of the cusp and the atrophic muscle fibers. Inflammatory cells are not present. Iron hematoxylin and eosin preparation.  $\times 180$ .

FIG. 11. Section of base of pocket of Case 6. Note the newly formed blood vessels. Iron hematoxylin and eosin preparation.  $\times 180$ .

bodies" was found to be 30.00 with a probable error of  $\pm 0.319$ , while the same index for cells with "late inclusions" was  $33.07 \pm 0.263$ . The difference between these two means is  $3.07 \pm 0.413$ . It is important to note that in every test applied, the variability of the cells with "early inclusions" was greater than that of the cells with "matured inclusion bodies." This fact seems to indicate that there is a much more rapid rate of increase in cell and nuclear volume during the early development of the inclusions than later on. Furthermore it seems probable that the cells with "late inclusions" have

TABLE II  
*Average Nuclear Cell Indices*

	Number of cells	Nuclear cell index	Standard deviation	Coefficient of variability
Normal duct cells	747	24.27		
Normal cells in infected ducts	597	20.44		
Cells with early inclusion bodies	150	$30.00 \pm 0.319^*$	$5.80 \pm 0.226$	$19.33 \pm 0.753$
Cells with late inclusion bodies	150	$33.07 \pm 0.263^*$	$4.77 \pm 0.186$	$14.42 \pm 0.562$

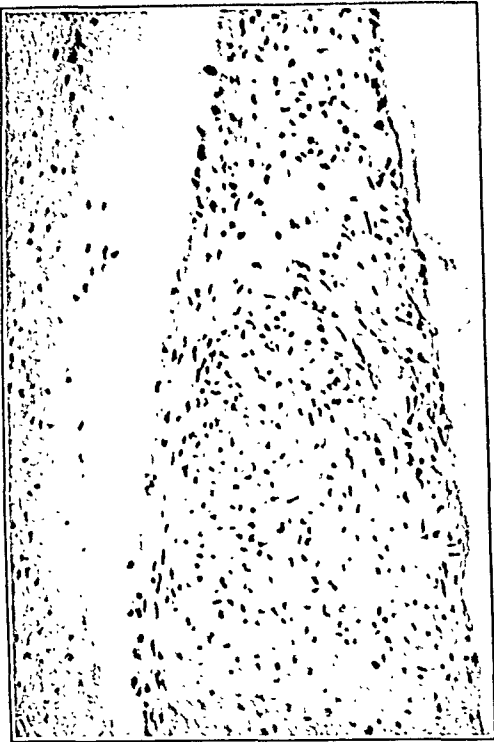
\* The difference of these two means is  $3.07 \pm 0.413$  and may therefore be regarded as significant.

reached the peak of their size increase and have become more or less stabilized with regard to volumetric relations.

These figures, with variability determinations, are given in detail in Table II. The scatter of the observations on nuclear cell index is illustrated by the frequency graph given in Text-Figure 2. It is of interest to note that the modal points of the two curves coincide although the means of these two sets of data are significantly different.

*Nucleocytoplasmic Index:* By the subtraction of nuclear volume from cell volume a figure was obtained which represents the volume of the cytoplasm for a given cell. An index of the proportion of nucleus to cytoplasm was then derived by dividing the volume of the nucleus by that of the cytoplasm and multiplying the result by one hundred. In the normal duct cells the nuclear volume was approximately one-third of that of the cytoplasm. The normal cells in the infected ducts showed, however, a decrease in this relation for the





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11



phagocytic cells. This does not imply that antibodies *per se* are unimportant; it does suggest the need of directing more attention to the cells themselves.

A further impetus to the enlargement of our concepts of tissue-immunity was furnished by Besredka<sup>2</sup> in his studies of local immunity. Although Besredka's interpretations of the mechanism of local tissue-immunity are not accepted by many, his ideas have stimulated much investigation and have aided in the clarification of a difficult problem of immunology. We also owe much of this clarification to the work of Gay and his associates,<sup>3, 4, 5</sup> who have greatly extended our knowledge of tissue-immunity and have done the most to correlate immune processes with histological changes in the tissues, thus furnishing a basis for the development of a field of histological immunology. In an extensive investigation of the problem of experimental streptococcic empyema, Gay and his collaborators have shown clearly that in this condition increased resistance of pleural cavities to streptococci is due primarily to an actual increase in numbers of tissue-macrophages in the wall of the thorax. Previous irritation of the pleural surfaces by the injection of such substances as gum arabic broth leads to the development or mobilization of large numbers of macrophages beneath the parietal pleura and these actively phagocytic cells ensure protection against large dosages when streptococci are later injected into the pleural cavity.

Opie<sup>6, 7, 8, 9</sup> has also made observations of the greatest importance in his studies of anaphylactic inflammation. He has demonstrated that when rabbits are injected intradermally with foreign proteins such as horse serum or crystalline egg albumin, much of the material is quickly demonstrable in the blood stream by precipitin tests, but "with repeated injection of the antigen the quantity of foreign protein that enters the blood stream diminishes and finally with advanced immunization none enters unless massive doses have been employed." Furthermore, in the immunized animal the foreign protein is fixed at the site of injection, a fact of significance because this is where the anaphylactic inflammation occurs. This inflammation is characterized by a rapid infiltration of polymorphonuclear leucocytes at the site of the inoculation, with edema and the deposition of fibrin. The small blood and lymph vessels are injured and thrombosis frequently occurs, leading, especially in the rabbit, to necrosis (Arthus phenomenon). Since a similar reaction occurs when antigen

## STUDIES IN TISSUE-IMMUNITY \*

### CELLULAR REACTIONS OF THE SKIN OF THE GUINEA PIG AS INFLUENCED BY LOCAL ACTIVE IMMUNIZATION

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The relatively unsatisfactory results of many years emphasis upon humoral factors in the defense against infectious diseases have gradually led to a reëxamination of some of the underlying mechanisms involved, and as a result the cellular reactions of immunity are now receiving more attention. This is not surprising in view of the many disappointments following attempts to secure preventive and curative serums, the transitory nature of passive immunity and the failure of protection at times in the presence of high concentrations of immune bodies in the serum. On the other hand, the permanency of active immunity, as after an attack of typhoid fever or small-pox, or from the administration of certain vaccines, offers possibilities of prevention far superior to those hitherto attained by the use of serums.

The emphasis upon cellular mechanisms of defense received renewed stimulus through the attention paid to the mesenchymal tissues by Aschoff<sup>1</sup> in the reticulo-endothelial system. The principal merit of Aschoff's work probably lies in its development of certain morphological aspects of defensive mechanisms; as a result, principles enunciated by Metschnikoff are now receiving more serious attention and are being revealed as fundamental in all considerations of the basic problems of immunology. Furthermore, accumulating evidence points increasingly to the close relationship between phagocytic cells, particularly macrophages, and antibody-formation, so that at present it is no radical concept that antibodies may be merely excess products resulting from the ingestion of antigenic substances by phagocytes. If this view is correct, the attempts to obtain serums or solutions of antibodies may be concerned mainly with by-products, the fundamental reaction actually occurring within the

\* Received for publication July 21, 1930.

of antigen and antibody were followed by a secondary infiltration of cells of inflammation into this area, the infectious agents should be even more effectively localized. This entire mechanism should then be considered as cellular and a type of tissue-immunity. Even in the presence of marked necrosis at the site of inoculation, if such a local reaction prevents the dissemination of the infectious antigen, the effect nevertheless is protective and an evidence of immunity. As Opie says, "the apparent susceptibility of the protected animal to local injury is a paradox explained by changes which serve to protect the organism as a whole." In other words, a scar from a furuncle is a small price to pay for the prevention of pyemia.

A phase of tissue-immunity which has received much attention within recent years is the problem of local immunity. In regard to this, we feel that too much emphasis has probably been placed on the localized nature of the immunity and on the rôle of antibodies in the phenomenon. Besredka defines local immunity as an "immunity without the obligatory participation of antibodies." Such a conception, if correct, would materially modify the usual conceptions as to the relative importance of cellular and humoral factors in immunity. Besredka's ideas are based, principally, on the evidence of an acquired immunity of local tissues, such as the skin and mucous membranes, in the absence of any significant degree of antibody-concentration in the blood serum. This, however, does not necessarily exclude the possibility of antibodies being within the cells or around them in the area of localized tissue-immunity; indeed, there is evidence that local concentration of antibodies may play a considerable part in the local tissue-immunity. Gay's definition of local immunity as an immunity "due to a locally superior mechanism for the disposal of a particular microörganism," seems to be much broader and more in conformity with the facts. This mechanism may or may not be associated with the action of antibodies, but until we know what function antibodies have within phagocytic cells and how quickly they may appear there, we cannot arbitrarily exclude them from participation in immune processes merely because they may seem to be of little significance as shown by the usual serological tests, especially in view of the increasing evidence that the usual site of antibody formation is in the individual cells of the mesenchymal tissues.

The demonstration of a localized type of immunity does not neces-

and antibody are simultaneously injected into the tissues of a rabbit, but not when antigen and normal serum are so injected, and since the same effect is observed when antiserum is injected into the tissues of a sensitized animal, Opie concludes that "anaphylactic inflammation occurs because antigen and antibody have met in the tissues." If we assume that at least part of this union occurs within tissue macrophages, the intracellular reaction may conceivably either give no effect or may give anaphylactic inflammation, depending upon the amount of antigen entering sensitized macrophages in a short period of time, or the relative concentration of intracellular antibodies available for the reaction with the antigen. In either case a too violent reaction could be followed by a certain degree of damage to the surrounding tissues with injury to capillaries, thus increasing their permeability for the plasma and cellular elements so prominent in inflammation, anaphylactic or otherwise. It is a reasonable hypothesis that at least some of the cutaneous reactions which we regard as anaphylactic or allergic may be due to an altered reactivity of these phagocytic cells, both as to rate of phagocytosis and rate of intracellular digestion of antigenic substances, as determined by the number of phagocytes available, their state of reactivity and the amount of antigen utilized per unit of time. Where anaphylactic inflammation occurs, the extent of the cellular infiltration may depend upon the degree of the chemotactic stimulus; at times the very violence of the reaction may overshadow the beneficent action, giving the "two-edged sword" effect of dissemination of the antigen.

To what degree can we regard this reaction as a mechanism of defense? The answer will be determined by the behavior of properly sensitized tissues to the entrance of pathogenic microorganisms. Probably the essential difference between the effects of infectious agents and of inert antigens is in the ability of the former to multiply and to invade tissues. If both multiplication and invasion could be prevented to a large extent because of a local union of antigen and antibody through an agglomerating reaction, such as agglutination or precipitation of the microorganisms or their products, the tendency to dissemination of the germs would be greatly lessened. If, in addition, an increased number of macrophages, especially ones sensitized by previous experiences with the antigen, were present in the area of invasion, phagocytosis should be quantitatively increased. Finally, if the reaction to injury through this local meeting

stage. The authors concluded that "the clasmatoocytes in large numbers diminish the virulence of the bacterial attack and also, by ingesting the polymorphonuclear leucocytes which contain staphylococci, they prevent a recurrence of bacterial activity."

An important study of the inflammatory reactions of the subcutaneous tissues of normal and immunized mice, using streptococci and pneumococci, was also made by Tsuda<sup>11</sup> in Lubarsch's laboratory. In normal animals this response varied with the degree of virulence of the microorganisms injected, but with weakly virulent germs the organisms remained at the site of inoculation, were quickly surrounded and phagocytosed by leucocytes and then encapsulated by connective tissue cells. There was thus no dissemination of the microorganisms through the adjacent tissues. With highly virulent microorganisms, however, there was an early injury to the tissues at the site of inoculation with very little phagocytosis of the germs, and with a consequent rapid dissemination of the latter throughout the surrounding tissues. In immunized animals both virulent and avirulent bacteria were quickly injured, as shown by evidences of degenerative changes such as swollen and poorly stained forms, inequality of size, etc. There was also a marked tendency to agglutination followed by active phagocytosis by the leucocytes and macrophages. This observation of agglutination *in vivo*, while not emphasized by Tsuda, is obviously of great significance. Tsuda states that "if the immunity is strong enough, the injected cocci show at the site of injection agglutination phenomena in the form of floccular clumps and aggregations of microorganisms."

More recently Imschenetzky<sup>12</sup> has shown that the application to rabbits of dressings saturated with isotonic salt solution or with staphylococcus antiviral solutions leads to a distinct inflammation in the subcutaneous tissues, with hyperemia, edema and an increased prominence of histiocytes and increased numbers of infiltrated leucocytes. Similar dressings saturated with a 1 per cent solution of trypan blue in salt solution led to the appearance of granules of dye in the histiocytes, but only after forty-eight-hour application of the dressings, and then only when there were evidences of slight injuries to the epidermis which had increased its permeability.

sarily imply the absence of protection elsewhere; resistance locally as well as generally is relative and a matter of degree. Nevertheless, by proper dosage, and by localized immunization, several investigators have shown that certain tissues may acquire an enhanced ability to resist invasion by microorganisms as compared with other tissues of the same individual. In such conditions of local immunity more evidence is needed as to the factors concerned, whether cellular, humoral, or both. Morphological evidence, particularly, is desirable, either of local dissolution or agglomeration of bacteria injected, or of an increased number of phagocytic cells in the area, or of increased metabolic activity of such cells. Curiously enough, although Besredka speaks of certain "receptive cells" which are dominant in localized areas of immunity, it seems that he has made little study of them from a histological viewpoint; nor have most of the other workers in this field given much consideration to this point.

Apparently the only histopathological study of the cellular reactions of the skin of the guinea pig to staphylococcus infection according to the methods of Besredka, is that of Freedlander and Toomey.<sup>10</sup> These workers made a detailed examination of the inflammatory response in the subcutaneous tissues of normal guinea pigs, and of ones previously treated with broth compresses and staphylococcus filtrates prepared according to the methods of Besredka. A definite localized protection was observed following the application of broth compresses, which protection persisted longer than twenty-four hours and less than seven days. The protection seemed to be non-specific and was correlated with histological changes in the subcutis where there was a significant increase in the numbers of clasmatocytes, fibrocytes and lymphoid cells following the application of sterile broth compresses for forty-eight hours. The inflammatory response to the subcutaneous injections of staphylococcus cultures in such animals was characterized by an infiltration of cells of inflammation in the subcutis, much more marked in degree than in normal animals similarly infected. Also, although polymorphonuclear leucocytes were the predominant cells in each case, they tended to degenerate in the control animals, whereas they retained their normal appearance in the broth-protected ones. In addition, in the latter animals there was an increased infiltration of small mononuclear cells and a greater prominence of clasmatocytes, with fibroblasts tending to organize the process at an early

In the denser areas the latter are elongated and compressed and at times resemble fibroblasts. Mallory's connective tissue stain, however, shows but slight increase in the amount of collagen, although there is a definite increase of collagen in the densest areas of macrophages. Mitotic figures are not seen in the regions of thickening, and the multiplicity of mononuclear types, from lymphocytes to typical macrophages, suggests that, as maintained by Maximow, the latter may be differentiated forms of cells of hematogenous origin, particularly lymphocytes or monocytes (see Fig. 1).

#### THE BEHAVIOR OF INDIA INK INJECTED INTO NORMAL SKIN AND INTO SKIN PREVIOUSLY IMMUNIZED AGAINST STAPHYLOCOCCI

The intradermal injection of 0.2 cc. of a 4 per cent suspension of India ink in sterile isotonic salt solution into a normal guinea pig was followed by a diffuse dispersion of the particulate material through the subcutis. Sections showed much of the ink caught along the collagenic fibrils, although some of it was engulfed by tissue macrophages. A moderate infiltration of polymorphonuclear leucocytes occurred at the end of twenty-four hours, but these did not engulf the particles of ink to any extent. When the same quantity of ink was injected into an area of skin of a guinea pig which had previously been given ten intracutaneous injections of plain peptone broth, the effects were not noticeably different from those observed in the normal animal. When a similar quantity was injected, however, into the skin of a guinea pig which had previously been immunized by ten intradermal injections of the killed staphylococcus vaccine, there was a distinct tendency for the ink to remain localized near the site of inoculation rather than to be dispersed through the subcutis. Fig. 9 shows the distribution of the ink in the skins of the three animals and Fig. 2 illustrates the mode of disposal of the ink particles by the macrophages of the subcutis. It is interesting that monocytoïd cells, lymphocytes and polymorphonuclear leucocytes show little tendency to engulf the particles of ink. These observations suggest that merely the presence of increased numbers of macrophages increases quantitatively the engulfment of particulate material and thus effectively aids the localization of such material after its inoculation. It is also possible that local hindrances to lymph flow may further prevent, to some extent, the dissemination of the particulate material.

## EXPERIMENTAL PROCEDURES

Our studies have been concerned with the cellular reactions of defense in the skin of normal guinea pigs and of others previously immunized by the intracutaneous injection of a staphylococcus vaccine. More than 100 different animals have been observed during the course of the investigation. A strain of staphylococcus aureus freshly isolated from a furuncle was used. For immunization, a twenty-four-hour growth on agar slants was suspended in 1 cc. of sterile 0.9 per cent salt solution and heated at 60° C for one hour. Two-tenths of a cubic centimeter of this vaccine was injected intradermally at daily intervals for ten days into the anterior abdominal wall of guinea pigs weighing from 200 to 300 grams, thus infiltrating an area of skin of approximately four square centimeters. The animals were then allowed to rest for twenty-five days in order to permit the skin to return to approximately normal conditions in so far as external appearances were concerned. Then these animals, and normal ones of the same size, were injected intradermally with 0.2 cc. of a living virulent culture of the organism, the growth from one agar slant again having been suspended in 1 cc. of sterile salt solution.

At intervals the guinea pigs were anesthetized and the areas of inflammation were excised after attaching the peritoneal surface to a cork frame by means of bamboo pegs. The tissues, usually averaging from one to one and a half centimeters in width, were immediately fixed in formol-Zenker fluid and subsequently embedded in celloidin and sectioned at 10 microns. The sections were stained routinely with Maximow's hematoxylin-eosin-azur II as well as with special stains such as Mallory's connective tissue stain, Goldmann's carmine stain and Gram's stain.

## EFFECTS OF THE INTRADERMAL INJECTION OF A STAPHYLOCOCCUS VACCINE UPON THE SKIN

Sections of skin taken twenty-five days subsequent to the ten intradermal inoculations of killed staphylococcus vaccine show the principal effect in the subreticular zone of the subcutis. Here there is an extremely marked increase in macrophages. In the looser areas many sizes and types of non-granular cells may be seen, varying from typical lymphocytes and monocytoïd forms to typical macrophages.



percentage value was but 24.40, that is to say the nuclear volume was only one-fourth of that of the cytoplasm (Table III).

Here again the infected cells with "early inclusions" exhibited greater variability than that seen in the cells with "late inclusions." The mean value of the nucleocytoplasmic index in the beginning in-

TABLE III  
*Average Nucleocytoplasmic Indices*

	Number of cells	Nucleocytoplasmic index	Standard deviation	Coefficient of variability
Normal duct cells	747	32.13		
Normal cells in infected ducts	597	24.40		
Cells with early inclusion bodies	150	$43.79 \pm 1.659$	$12.30 \pm 1.173$	$28.09 \pm 2.680$
Cells with late inclusion bodies	150	$50.16 \pm 1.500$	$11.12 \pm 1.061$	$22.17 \pm 2.115$

fections proved to be  $43.79 \pm 1.659$ ; while at the later stage it was  $50.16 \pm 1.500$ . Although the difference of the mean values was nearly 7 per cent, the coefficient of variability was so high that the significance of the difference is problematical. The standard deviation was high in both cases.

Evidently, therefore, the relation of the size of the nucleus to the size of the cell is, in infected cells, a more constant proportion than that of the nucleus to the cytoplasm.

TABLE IV  
*Average Inclusion-Nuclear Indices*

	Number of cells	Inclusion nuclear index	Standard deviation	Coefficient of variability
Cells with early inclusion bodies	150	$17.07 \pm 0.396^*$	$7.19 \pm 0.280$	$41.88 \pm 1.631$
Cells with late inclusion bodies	150	$30.89 \pm 0.362^*$	$6.58 \pm 0.256$	$21.16 \pm 0.824$

\* The difference of these two means is  $13.82 \pm 0.537$  and may therefore be regarded as significant.

## GENERAL RESULTS

The inflammatory response to the living staphylococci was markedly different in the skins of the two groups of animals. In the normal guinea pigs the intradermal inoculation led to a serosanguineous inflammation which spread as a diffuse cellulitis through the subcutaneous tissues and frequently led to the death of the animal in from eighteen to twenty-four hours. In the previously immunized ones, however, the intradermal injection was followed by a localized small area of suppuration which tended to ulcerate and heal with no serious consequences to the host. It is evident from these differences in the reactivity that the intradermal injections of the killed culture of staphylococcus led to an increased resistance of the skin to the later injection of the living virulent organisms. Examination of the tissues confirmed the above observations and in addition suggested an explanation for the increased resistance of the skin of the immunized animals.

## EFFECTS OF INTRADERMAL INJECTIONS WITH LIVING STAPHYLOCOCCUS AUREUS SUSPENSION UPON NORMAL GUINEA PIGS

The inflammatory response is well developed within six hours, as shown in Fig. 5*a*. The principal finding in the skin of the normal animal at this stage is edema of the subcutis with separation of the collagenic fibrils and a beginning infiltration of cells of inflammation. Polymorphonuclear leucocytes comprise the vast majority of the incoming cells and these are actively phagocytosing the staphylococci as shown in Fig. 3. In spite of this fact, however, the staphylococci are diffusely spread along the subcuticular tissue in the form of a developing cellulitis. There is no evidence of any localizing tendency of the microorganisms, as shown in Fig. 10, nor are there evidences of injury to the bacteria, such as numerous swollen or distorted forms or frequent Gram-negative cocci. The infection is predominantly dispersive and generalizing. In the later stages, twelve, eighteen, and twenty-two hours, the picture is similar except for the greater infiltration of cells of inflammation, principally polymorphonuclear leucocytes (Figs. 6*a*, 7*a* and 8*a*). In spite of the activity of the microphages in ingesting many staphylococci, the infection progresses; in other words, the natural resistance is inadequate as a defensive mechanism.

## REACTIONS IN SKIN PREVIOUSLY IMMUNIZED BY INTRADERMAL INJECTIONS OF A KILLED STAPHYLOCOCCUS VACCINE

The inflammatory response to the injection of 0.2 cc. of the same suspension of living staphylococci into the skins previously immunized is markedly different. As may be seen in Figs. 5*b*, 6*b*, 7*b* and 8*b* the principal difference is one of degree. At the six-hour stage there is an enormous infiltration of cells of inflammation in the subcutis of the immunized skin, much more abundant than in the normal animal at this stage. Furthermore, there is a qualitative difference in that there are many more lymphocytes and monocytoïd cells present. These cells tend to become massed around a region of marked bacterial concentration and here there are many evidences of necrosis of the cells of inflammation, with accompanying hemorrhage and even thrombosis of the capillaries. Outside this area there are very few staphylococci to be seen; the infection is definitely localized and non-dispersive. A point of interest and probably of great importance is that in the area where the staphylococci are massed they are not present as individual organisms, but occur extracellularly in clumps and clusters, large and small, even in the six-hour stage. The appearance is that of agglutination *in vivo* (Figs. 11 and 12). It is around these clumps of staphylococci that the infiltration of leucocytes is densest, with the microphages nearest to the bacteria containing large masses of microorganisms. Here also the macrophages contain countless numbers of cocci, as shown in Fig. 4. It is obvious that the infection is localized to the immediate vicinity of the site of inoculation and that the acquired resistance is adequate as a defensive mechanism.

### DISCUSSION

It is an interesting fact that Metschnikoff showed in 1884<sup>13</sup> that the principal difference in the reaction to the subcutaneous injection of virulent anthrax bacilli in normal rabbits, and in others previously immunized, lay in the greater degree of phagocytosis in the latter. He noted that within a few hours after the injection of the organisms into the normal animal there was an exudation rich in fluid and poor in leucocytes, in spite of the fact that the blood vessels in the vicinity were distended with blood and therefore could not be considered as unable to bring the leucocytes to the infected area. In the

vaccinated rabbits, however, there was an exudate rich in leucocytes at the site of inoculation and these were actively phagocytosing the bacilli. Metschnikoff concluded that the essential difference depended upon the sensitiveness of the leucocytes which exhibited a negative chemotaxis in the normal rabbit, but a marked positive chemotaxis in the immunized ones.

The effects of the entrance of pathogenic bacteria into the skin will obviously depend upon their ability to gain a foothold, multiply and disseminate throughout the body. There is no doubt that microorganisms vary in their ability to adapt themselves in the animal's body, this probably being a property inherent in the microorganisms themselves. The growth energy of certain highly virulent strains may possibly be so pronounced at times that dissemination occurs before the body cells or fluids can mobilize to hinder this dissemination.

Under more usual conditions the ability to adapt, multiply and disseminate is prevented by the defensive forces of the body. These may be both cellular and humoral. The early mobilization of phagocytic cells at the site of bacterial infection may serve to restrain the rapid increase in numbers of bacteria and in most cases the rate of engulfment by the macrophages may exceed the rate of multiplication of the bacteria. This, plus probable mechanical hindrances from the accumulation of fibrin and cells around the region of infection, will effectually localize the latter. In any event, the infection is obviously localized and the dissemination of the microorganisms is prevented.

In this localization of the bacteria at the site of inoculation the exact mechanism is still somewhat obscure. Are the organisms localized because of the intense infiltration of cells and fluids which mechanically hinder the further spread of the bacteria in the sense of the allergic inflammation of tuberculosis as conceived by Krause,<sup>14</sup> or are the organisms first localized by a mechanism of immunity and secondarily encapsulated because of the infiltration of cells of inflammation? The work of Opie would suggest the latter explanation as better fitting the facts and our results indicate the same probability. For example, the demonstration in the immune guinea pig within six hours after the injection of distinct extracellular clumping of the staphylococci with an accompanying failure of dissemination of the microorganisms strongly suggests a primary localiza-

tion of the bacteria through their reaction with antibody. Furthermore, no such tendency at any stage was noticed in the normal animals so that it does not seem probable that the bacterial masses are colonies growing in the tissues. It is of course possible that the clumping of the bacteria in the immune animal may be due to mechanical interferences with lymph flow which, with the dense layer of macrophages surrounding them, may keep the staphylococci localized. This conception seems less probable, however, when one sees how easily the polymorphonuclear leucocytes infiltrate the area and surround the masses of bacteria. We suggest, rather, that there is an actual antigen-antibody reaction in the tissues; as a result of this reaction chemotactic substances are formed which quickly lead to a pronounced infiltration of cells of inflammation, which are both quantitatively and qualitatively different from those responding in the normal animal (anaphylactic inflammation). Added to this, also, is the evidence that phagocytosis by the histiocytes is more abundant quantitatively than in the normal animals, in addition to the greatly increased number of histiocytes available in the former. The experiments with India ink, described above, strongly suggest that an increase in histiocytes alone tends to localize particulate materials, but whether this is the result of increased phagocytosis or of a mechanical barrier remains uncertain. In active infection, however, it is probably the summation of all of these forces, specific as well as non-specific, that ensures an effective resistance against extension of the infection.

Additional support to the conception of the specific reaction is furnished by the experiments of Mudd, Lucké, McCutcheon and Strumia,<sup>15, 16</sup> which show the correlation between agglutination, cohesiveness, opsonization and phagocytosis of bacteria. If such correlations also obtain within the body, the evidences of agglutination and phagocytosis in our experiments may furnish a further clue to the function of immune bodies. Agglutination of the staphylococci may have furthered the tendency to their localization near the site of inoculation; their coalescence into small masses may also have increased the defensive efficiency of the phagocytes, both leucocytes and macrophages, since more microorganisms per phagocyte may be ingested following chance contacts than if the organisms were single and dispersed. Furthermore, if the agglutinating tendency and increased cohesiveness have an opsonizing effect, phagocytosis

will be further increased. In this connection it is interesting to recall the earlier conceptions of Bull <sup>17</sup> in his statement that "the degree of agglutination and opsonization of bacteria within the animal body is inversely parallel to the infectiousness of the bacteria for the host."

The relative importance of non-specific and specific agencies in localized tissue-immunity is difficult to evaluate. Certainly non-specific factors may be sufficient to protect against many multiples of the lethal dose of an infectious agent, as was well shown in the experiments of Gay and his collaborators in the study of streptococcic empyema. Rivers and Tillett,<sup>18</sup> and Mallory and Marble,<sup>19</sup> found that the injection of plain meat infusion broth protected the skin of rabbits against later injections of streptococcus and staphylococcus. Miller <sup>20</sup> also demonstrated a definitely increased resistance of the skin of guinea pigs to staphylococcus, and of rabbits to streptococcus infections following previous treatments with dressings saturated with bouillon and peptone water. In all of these experiments, however, the results do not prove that such non-specifically increased resistance would have been adequate for larger infective doses of the microorganisms used, or that even better protection might not have been secured by the aid of specific modes of treatment.

The mechanism of the non-specifically increased resistance may be explained in at least two ways: first, in a local infiltration of leucocytes and bactericidal substances which may more effectively dispose of the infecting organisms later injected, or second, in a stimulation of the local fixed-tissue cells to increased functional activity. Evidence for the second possibility is suggested by Katsunuma and Sumi <sup>21</sup> in their observation that when rabbits were injected subcutaneously with a suspension of staphylococci on one side, and with a similar quantity of salt solution on the other side, followed a few hours later by an injection of an emulsion of staphylococci into both sides, the exudates collected showed much more active phagocytosis on the side previously injected with the staphylococci.

The evidence is quite convincing, however, that non-specific factors are not exclusively responsible for the increased resistance in localized tissue-immunity. For example, Gay and his associates found in testing the protection of the pleural cavity to streptococcus infection that "the degree of protection acquired by the repeated administration of living streptococci subsequent to broth or aleu-

ronat preparation of the cavity is markedly increased over the protection obtained by a single injection of the broth or aleuronat." More recently Clark <sup>22</sup> has shown the importance of specific mechanisms in experiments concerning tissue-immunity to pneumococcus infections of the pleural cavity of rabbits. With this organism no protection was obtained following the injection into pleural cavities of substances which had previously been shown to protect completely against infection with the streptococcus through the mobilization of macrophages. When the pneumococci, however, were treated with immune serum before being injected into the prepared pleural cavity, there was marked protection in cavities containing an exudate rich in mononuclear cells and with a pleural wall fortified by increased numbers of macrophages. These experiments clearly show the significance of specificity; the opsonization of the organisms is thus an important feature which favors increased phagocytosis by the macrophages.

The specific factor in our experiments seems to be mainly an agglomerating force which we believe is true agglutination *in vivo*. Certainly the microorganisms occur in clumps in the immune skins and are not thus seen in the normal skins. It is possible that the increased number of macrophages present in the immunized skins interferes with lymph flow in a mechanical fashion and thus encourages approximation of groups of cocci; experiments now in progress may throw further light on this phase of the problem. If we assume that the agglomeration of the staphylococci is specific agglutination *in vivo*, we have concrete evidence of a fundamental immunological rôle of such antibodies in aiding the local fixation of antigen in tissues. Opie's experiments clearly prove this for precipitins and he has shown that such precipitates are strongly chemotactic for polymorphonuclear leucocytes which probably destroy the injected antigen by intracellular digestion. We believe that this concept is of the utmost importance when applied to the fate of living antigen introduced into the tissues. In our experiments the facts are clear that the staphylococci disseminate diffusely throughout the subcutaneous tissues of the normal animals with no tendency to agglomerate or agglutinate. On the other hand, in the immune animals the tendency to agglomerate is noticed as early as six hours after injection of the microorganisms. Coincidentally, there is a localization of the staphylococci near to the site of

inoculation, with a pronounced infiltration of cells of inflammation, both granulocytes and agranulocytes, around the bacteria. Local injury to this area occurs, but the animal itself is protected. In the words of Opie "vital organs are protected at the expense of local injury."

### SUMMARY AND CONCLUSIONS

This paper describes histopathological studies of the skin and subcutaneous tissues of the abdominal wall of normal guinea pigs and of ones previously immunized by intracutaneous injections of a staphylococcus vaccine, all infected by the intracutaneous injection of a live virulent culture of staphylococcus aureus. The inflammatory responses were markedly different in the two groups. In the normal animals the inflammation was characterized mainly by an infiltration of polymorphonuclear leucocytes which actively phagocytosed the microorganisms. In spite of this the staphylococci showed no tendency to localize, but disseminated throughout the subcutaneous tissues in the form of a cellulitis.

In the previously immunized animals, however, the staphylococci tended to remain localized near the site of inoculation where they were seen agglomerated in bacterial masses of various sizes, presenting the picture of a genuine agglutination *in vivo*. Coincidentally the infiltration of cells of inflammation led to further localization of the microorganisms so that only a localized area of necrosis resulted.

The previous immunization by intracutaneous injections of the staphylococcus vaccine was followed by a marked thickening of the subreticular layer of the subcutis, due mainly to increased numbers of tissue macrophages having been either activated or produced. Evidence is presented that many of these are derived from agranulocytes of the blood. These macrophages were actively phagocytic for the live staphylococci and furnished an effective barrier against extension of the infection.

The immunity secured by the above procedures is predominantly cellular in type with the tissue-macrophages playing the dominant part, due to increased numbers and also probably to increased metabolic activity. In addition, localization of the microorganisms by the action of agglutinating or opsonizing antibodies is suggested as of primary importance in preventing the dissemination of the infectious agent. The combination of humoral and cellular mechanisms ensures an adequate resistance against the bacterial invaders.



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## DESCRIPTION OF PLATES

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### PLATE 140

FIG. 1. Drawing made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, showing the effects of intracutaneous injections of a staphylococcus vaccine upon the subcutis of a guinea pig. Note the types of agranulocytes, varying from typical lymphocytes and monocytoïd forms to typical macrophages. The tissue was excised twenty-five days after the end of the period of immunization. Stained with hematoxylin-eosin-azur II.

FIG. 2. Oil immersion drawing made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, stained by Goldmann's carmine method. This illustrates the mode of disposal of particulate material by macrophages in the subcutis of the guinea pig after intracutaneous immunization by ten injections of staphylococcus vaccine. This animal was injected intradermally with 0.2 cc. of a 4 per cent suspension of India ink in 0.85 per cent sodium chloride solution, and the skin excised twenty-four hours later. Note the active ingestion of ink particles by the macrophages and the absence of such ingestion by the lymphocytes, polymorphonuclear leucocytes and monocytoïd cells. Note also the adherence of small particles to fibrils of collagen.

FIG. 3. Oil immersion drawing, made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, stained with hematoxylin-eosin-azur II. This drawing is from the subcutis of a normal guinea pig six hours subsequent to the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococcus aureus. Note the active ingestion of staphylococci by the polymorphonuclear leucocytes, which cells are almost the only ones responding at this stage of the inflammation. Note also the diffuse distribution of these cells and of the microorganisms.

FIG. 4. Oil immersion drawing, made at the level of the substage with the aid of the camera lucida, Leitz Oc. 4, Obj. 2 mm. apochromatic, from section stained with hematoxylin-eosin-azur II. From the subcutis of a guinea pig previously immunized intracutaneously by injections of staphylococcus vaccine, and infected intracutaneously with 0.2 cc. of a living virulent suspension of staphylococci. Tissue excised eighteen hours after the infection shows many macrophages actively phagocytosing staphylococci. The infection remained localized to the site of inoculation.

*Inclusion-Nuclear Index:* An index of the volumetric relation of the size of the inclusion to that of the nucleus was obtained by dividing the volume of the inclusion by that of the nucleus and multiplying the resulting figure by one hundred. It was found that cells with "early inclusions" showed an index of but  $17.07 \pm 0.396$  while in those with "mature inclusion bodies" the index was  $30.89 \pm 0.362$ . The difference here was of unquestionable significance. Again the cells with "early inclusion bodies" proved themselves to be more variable in this relation than their successors (see Table IV).

### DISCUSSION

These results are interesting from several points of view. The use of quantitative methods in which definite measurements are treated mathematically eliminates the personal equation in making observations. Though laborious they bring to light cellular changes which otherwise would remain undetected. For example, cells not containing inclusions but situated near others which do, appear to be normal, as judged by their tinctorial properties and mitochondria. Unconscious comparison with the greatly hypertrophied inclusion-laden cells, by the observer, gives the distinct impression that they are smaller than usual (personal equation); but measurements show that on the contrary they are themselves slightly enlarged — a modification which probably might not have been noticed unless quantitative methods had been employed. We do not know what causes this change. The cells are not responding to the virus in the usual way, for this reaction is peculiarly selective involving isolated individual cells and not masses of them. The neighboring cells with inclusions, with which they were compared, themselves represent a response to the virus, probably of several days standing. It does not seem possible that the infection, whatever it may be, passes from the greatly hypertrophied cells to those in the vicinity, for the surrounding cells do not show "early inclusions." All cells with inclusions in an individual duct seem to be in approximately the same stage of development. The chief difference between an infected duct and a non-infected one is that the former contains cells which have been injured as a result of their reaction to the virus. It is possible that these cells while still living or even after death give off a substance, or substances, which cause the slight hypertrophy of the neighboring cells which we have reported.

PLATE 141

- FIG. 5. Photomicrographs of sections through the skin and entire abdominal wall of (a) normal guinea pig and (b) intracutaneously immunized guinea pig. The tissues were excised six hours following the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococci into each animal. In (a) there is slight edema of the subcutis with beginning infiltration of cells of inflammation. In (b) note the increased thickness of the subcutis and the more intense infiltration of cells of inflammation.  $\times 25$ .
- FIG. 6. Photomicrographs of sections through the skin and entire abdominal wall of two other guinea pigs treated as described in Fig. 5. The tissues were excised eleven to twelve hours following intracutaneous infection.  $\times 25$ .
- FIG. 7. Photomicrographs of sections through the skin and entire abdominal wall of two other guinea pigs treated as described in Fig. 5. The tissues were excised eighteen hours following intracutaneous infection.  $\times 25$ .
- FIG. 8. Photomicrographs of sections through the skin and entire abdominal wall of two other guinea pigs treated as described in Fig. 5. The tissues were excised twenty-two hours following intracutaneous infection.  $\times 25$ .

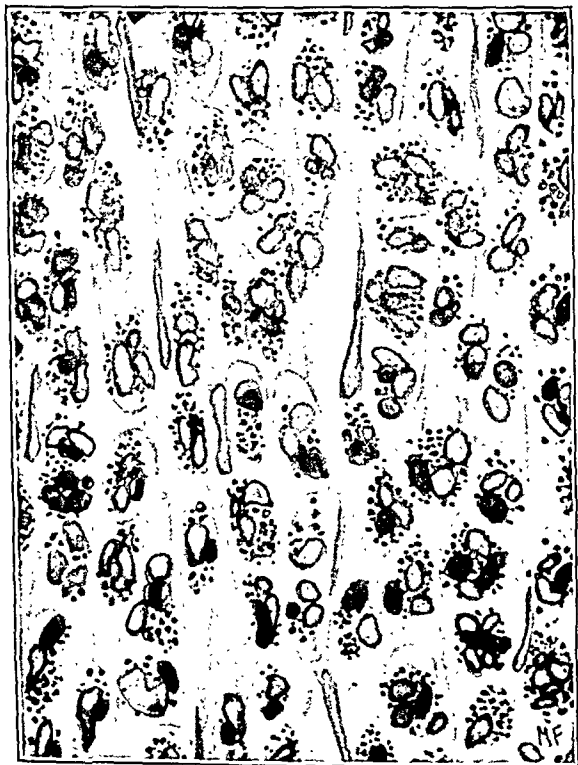
Note in all instances the more intense inflammatory response in the subcutis of the immunized animals.



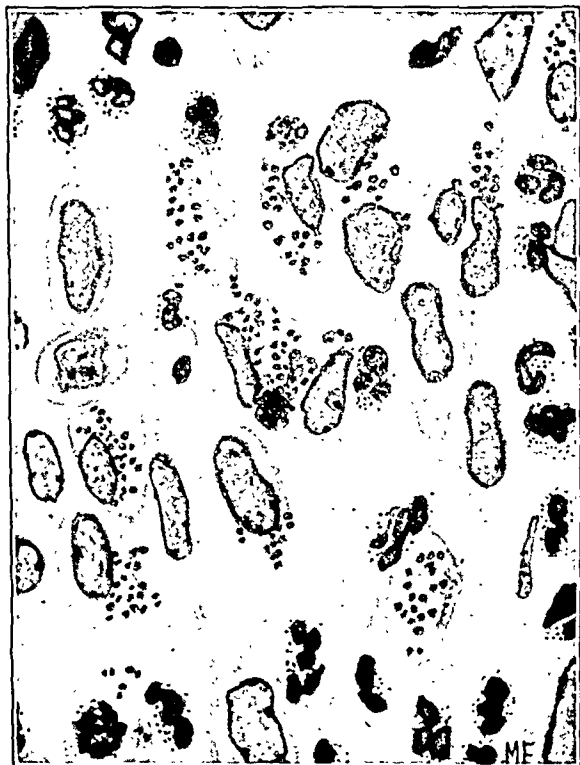
I



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3



4

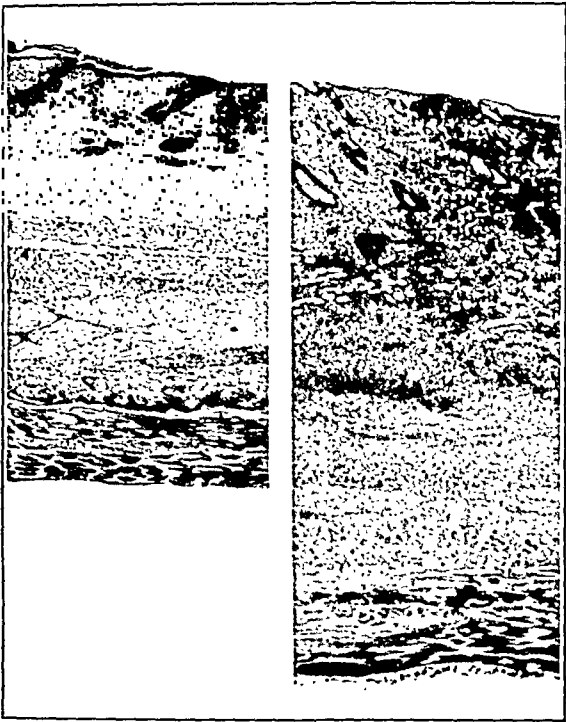
# PLATE 142

FIG. 9. Photograph of celloidin-embedded tissues of the entire thickness of the abdominal wall from three guinea pigs, each having been injected intracutaneously twenty-four hours before with 0.2 cc. of a 4 per cent suspension of India ink in 0.85 per cent sodium chloride solution. (a) Normal. (b) Previously given ten intracutaneous injections of sterile peptone broth. (c) Previously immunized by ten intracutaneous injections of the staphylococcus vaccine, the last injection given eleven days before. Note the dissemination of the suspension of ink along the subcutis in Nos. 1 and 2 and the tendency to localization of the ink near the site of inoculation in No. 3.

FIG. 10. Photomicrograph of the subcutis of a normal guinea pig six hours following the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococcus aureus. Note the diffuse distribution of the microorganisms with the tendency to spread along collagenic fibrils and to occur singly or in small clusters.  $\times 1400$ .

FIG. 11. Photomicrograph of the subcutis of a previously intracutaneously immunized guinea pig six hours following the intracutaneous injection of 0.2 cc. of the same suspension of staphylococci injected into the animal shown in Fig. 10. Note the greater tendency to concentration of the microorganisms, with the occurrence of the staphylococci extracellularly in coalescing clumps and clusters. The appearance is strongly suggestive of agglutination *in vivo*.  $\times 1400$ .

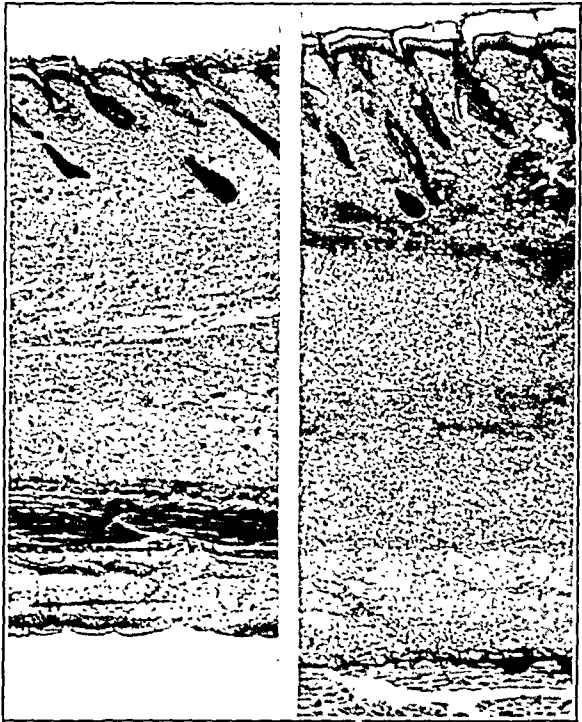
FIG. 12. Photomicrograph of the subcutis of a guinea pig previously immunized intracutaneously, twenty-two hours following the intracutaneous injection of 0.2 cc. of a living virulent suspension of staphylococcus aureus. Note the large masses of extracellular staphylococci, in clusters large and small, suggesting agglutination *in vivo*. These masses were completely encircled by a dense accumulation of cells of inflammation, principally polymorphonuclear leucocytes and macrophages. There was no tendency for the infection to disseminate beyond this area of microorganisms.  $\times 1400$ .



a

b

5



a

b

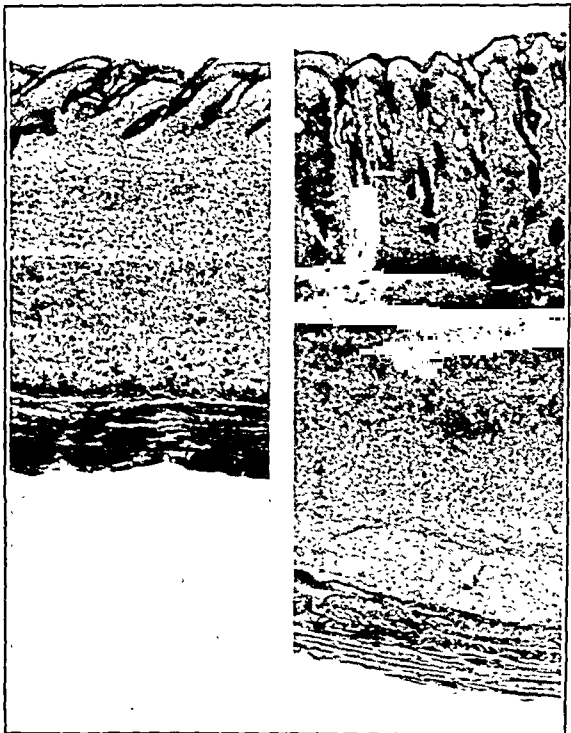
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a

b

7



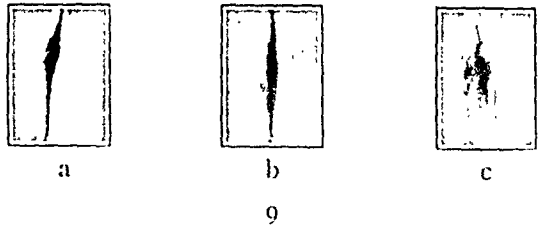
a

b

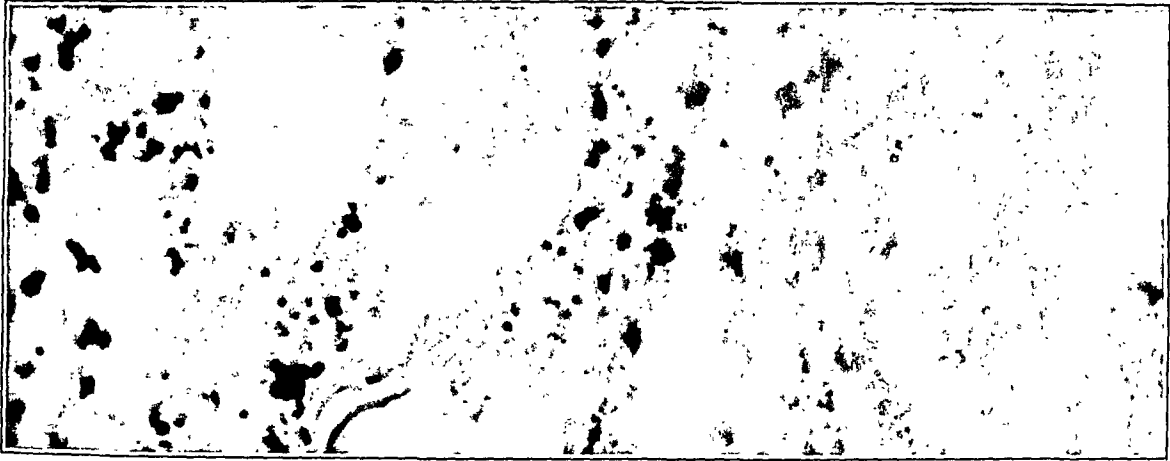
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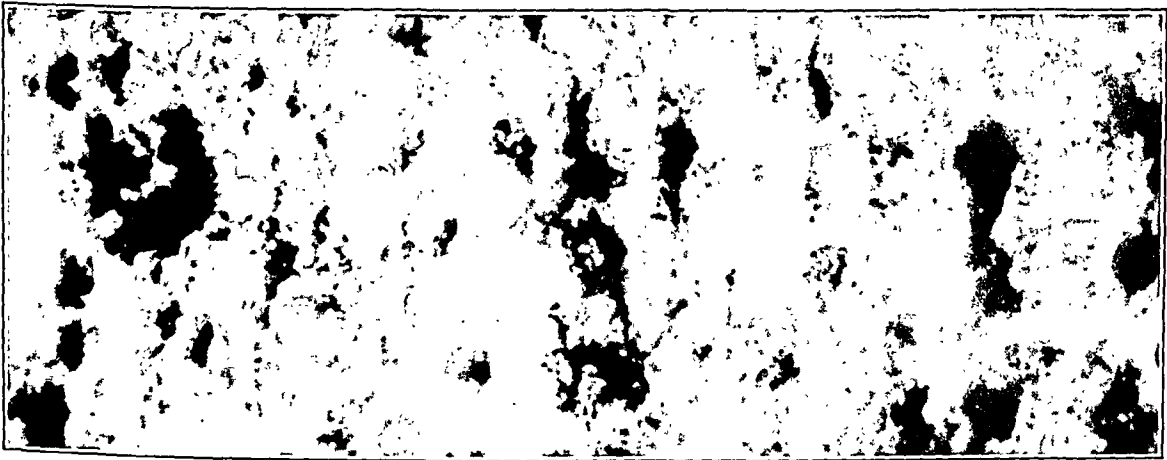




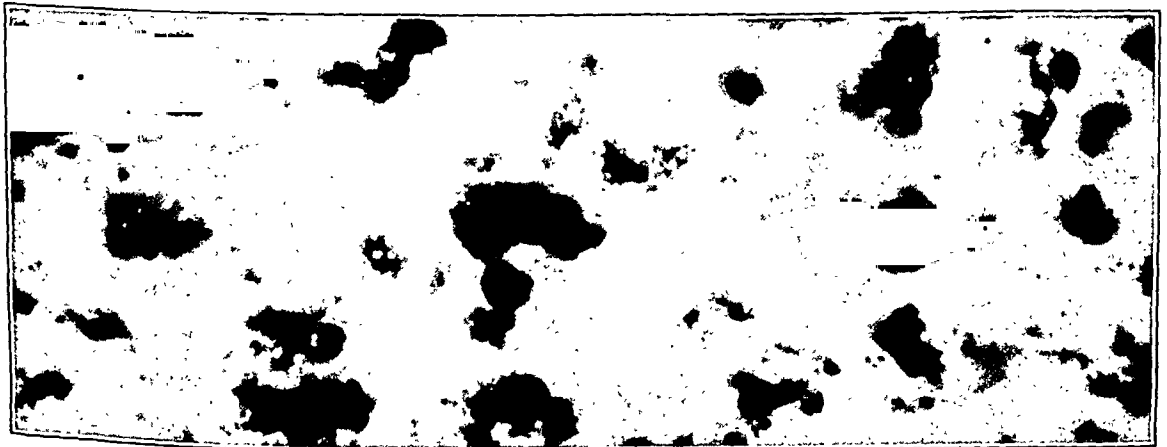
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10



11



12

beneath the ependyma, where it is possible that they represent a normal reservoir of young microglia cells (Penfield<sup>17</sup>). They are also seen in isolated scattered collections through the brain substance. Although it is impossible to demonstrate the presence of a parasite, it is equally impossible to say that these lesions are not either recent or healed lesions of parasitic origin.

2. The parasitic disease described by Wright and Craighead,<sup>18</sup> Levaditi *et al.*,<sup>19</sup> Twort and Archer,<sup>20</sup> Bender<sup>21</sup> and McCartney.<sup>22</sup> The lesions of this disease consist of round cell infiltrations of the meninges, perivascular cuffs and collections of cells beside vessels, necrotic areas in which the parasite may be seen, and cysts containing the "spores" of the organism. In the present series, lesions of these types were frequently met, but in only one brain (that of a rabbit which died of an apparently typical herpetic encephalitis on the seventh day after inoculation) was it possible to demonstrate the parasite itself.

3. Holes and spaces, occasionally with a small nucleus on their extreme edge, containing a hyaline material which stains with mucicarmine and with carbol fuchsin-formaldehyde. In the series under consideration, this picture occurred in two brains, one uninoculated, the other neurovaccinal. But in nearly all of them, spaces were seen which differed only in that they were empty. In brains fixed in formalin, the above description holds exactly. In Zenker-fixed material, however, the holes were smaller and less regular, and what mucoid staining there was was diffuse. This finding is included for the sake of completeness and because of its resemblance to the mucoid degeneration of Buscaino which has been discussed by many writers, including Ferraro.<sup>23</sup> It was not correlated with any other changes, and in view of the action of formalin on cerebral lipoids (Weil<sup>24</sup>), the extent of its pathological significance is not clear.

#### MATERIAL AND TECHNIQUE

*Neurovaccinia:* The strain of vaccinia used was one derived from calf lymph and "adapted" to the rabbit brain by Dr. Fei-fang Tang by testicular passage. I was enabled to use it through the kindness of Dr. Ward.

Six "normal" rabbits were injected intracerebrally with 0.5 cc. of a 5 per cent triturate in saline or hormone broth of the brains of

A COMPARATIVE HISTOLOGICAL STUDY OF ACUTE MENINGO-  
ENCEPHALITIS PRODUCED IN RABBITS BY THE VIRUSES  
OF NEUROVACCINIA AND HERPES SIMPLEX \*

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The purpose of this investigation was to make an attempt to compare the histological picture of neurovaccinal meningo-encephalitis in rabbits with that produced by the herpes virus, making allowance for the many apparently pathological changes which are found in the brains of uninoculated laboratory rabbits.

Levaditi, Harvier and Nicolau <sup>1</sup> in 1922, and more recently Turnbull and McIntosh,<sup>2, 3</sup> have published accounts of the histology of the neurovaccinal disease in which they agree that the major pathological changes are found in the meninges. Levaditi and Nicolau <sup>4</sup> describe a mononuclear infiltration of the pia, preceded in the first two days after inoculation by a polymorphonuclear infiltration. They describe the meningeal vessels as surrounded by mononuclear cuffs, the condition being very marked in the septa. According to them, the initial lesion is a true vaccinal pustule in the dura mater. No account of inclusion bodies in neurovaccinal encephalitis has been found.

Da Fano <sup>5</sup> in 1923 described the general picture of herpetic meningo-encephalitis in rabbits with great thoroughness, but he did not then give a very clear account of the intranuclear inclusions. These had been noted by several workers, Levaditi *et al.*,<sup>1, 6</sup> Luger and Lauda,<sup>7, 8</sup> and especially Lipschütz.<sup>9, 10, 11</sup> Further studies on this subject have been made by Goodpasture,<sup>12, 13, 14</sup> and by Cowdry,<sup>15</sup> and a summary of the work up to 1928 is given in Rivers' "Filterable Viruses" by Cowdry.<sup>16</sup>

The variety of apparently pathological lesions which are seen in the brains of "normal" uninoculated laboratory rabbits is notorious. They may be classified roughly as follows:

1. Round cell infiltrations unassociated with any inflammatory or necrotic changes or with any demonstrable parasite. These occur

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Giemsa, Mallory's phosphotungstic acid hematoxylin and Mayer's mucicarmine, and with carbol fuchsin one-fourth strength decolorized with strong formalin (see Wright and Craighead<sup>18</sup>). Most of the sections were also stained with Weigert's fibrin stain, in search of organisms.

Frozen sections, 15 microns thick, from the brains that were fixed in formalin were stained by Spielmeyer's iron hematoxylin method to show changes in the myelin sheaths.

### MACROSCOPIC CHANGES

The degree of vascular engorgement of the meninges differed more between the different brains of one disease than between the two diseases in general. In three of the brains small hemorrhagic lesions were visible at the site of injection.

### MICROSCOPIC EXAMINATION

*The Meninges:* In both diseases a marked meningitis was present in all cases, extending over any part of the brain and cord, and in the pial infoldings and septa. A perivascular arrangement of the cellular elements was often evident, but the greater part of the infiltration was spread diffusely through the pia-arachnoid.

Vasodilatation and hemorrhage were marked over the cerebellum. In three such hemorrhages (two vaccinia and one herpes) which had broken into the cerebellum, reaching the granular layer, there were masses of tissue containing all the elements of bone marrow (blood cells of all kinds, eosinophiles, large vacuolated fat cells, giant cells and many mitoses).

In 50 per cent of the vaccinia cases there were patches of nuclear degeneration scattered through the meninges, recalling Levaditi's statement that a true vaccinal pustule forms. This finding occurred rarely in the herpetic brains.

The relative amounts of fibrinous exudate and cellular reaction varied in different brains, and in different parts of the same brain. The predominant cell was a small round cell, probably a lymphocyte. In all cases, but in the vaccinal much more than in the herpetic, there were large numbers of polymorphonuclear cells with eosinophilic granules. Occasional plasma cells were seen, but there were not nearly so many as in more chronic cases of herpes.

rabbits that had died of the disease. Injections were made under light ether anesthesia, the skin and bone over the frontal lobe being pierced with a heated iron point, and the injection being made directly into the brain. In order to avoid mechanical injury to the brain, two other rabbits received similar injections into the cisterna magna, between the atlas vertebra and the occiput.

The typical course of the disease was as follows: The temperature rose on the second or third day to between 104° F and 106° F, falling rapidly to below normal shortly before death. The animals became weak and thin, and sometimes tremulous. No fits or definite paralysees were observed, except in one of the cisterna rabbits which developed a flaccid paralysis of the right fore limb immediately after the operation. Shortly before death most of the animals developed head retraction: they died with the head drawn right back and the back arched by a vigorous contraction of the spinal muscles, thus calling to mind the basal meningitis of man.

Death occurred on the third day in two rabbits, and on the fourth day in the other four rabbits. From time to time the brains of control rabbits that had received similar injections were ground up and injected intradermally into the shaved skin of other rabbits. Typical skin lesions were produced with dilutions of 1:1000 of the 5 per cent suspension.

*Herpes:* Brains of four rabbits treated as above, but with herpetic material, were examined. Of these, two died on the fourth day, one on the fifth and one on the seventh. All four showed the classical symptoms of herpetic encephalitis: salivation, teeth grinding, hyperexcitability and epileptiform fits, etc., — a condition which contrasts strongly with the neurovaccinal disease, in which the meningitic side of the picture predominates.

Brains were removed immediately after death, except in one instance, when the rabbit died in the night. Animals which were obviously moribund (*i. e.*, with head retraction, subnormal temperature, etc.), were etherized.

After removal, brains and cords were placed immediately in Zenker's fluid (with acetic acid) or in isotonic formol saline (10 per cent formaldehyde, neutralized with MgO). After one or two hours of preliminary hardening, they were cut in slices and returned to the fixative. Transverse paraffin sections from an average of seven planes were examined. All were stained with hematoxylin and eosin,

At the beginning of the reaction to the virus the duct cells are unquestionably alive and engaged in the transport of fluids. It is difficult to explain why some respond and develop intranuclear inclusions while the vast majority do not. The cells affected are not particularly young, for mitotic figures are found only with the greatest difficulty, in fact only one dividing cell was seen in several hundred glands examined. This instance was in a normal secretory duct of a gland from a guinea pig aged three weeks. Another factor which is said to promote cellular response to virus action is mechanical injury, but there is no evidence that such has occurred. From the physiological point of view there are no observations which would indicate that neighboring cells in the ducts may be normally in distinctly different metabolic states. Yet, as we have stated, some cells respond and others do not. It can hardly be a case of the avenue of approach of the virus, which would appear in many instances at least to be by the blood stream, because contiguous cells are evidently supplied approximately equally.

When this barrier against virus action which is effective for most of the duct cells is broken down, the cells apparently invariably succumb. There are no indications that affected cells ever recover. They pass through a series of changes which we have studied quantitatively and qualitatively. In the hypertrophy which takes place the nucleus at first leads the cytoplasm. Thus, in cells with "early inclusions" the volume of the nucleus has increased 75 per cent and that of the cytoplasm only a little over 30 per cent.

The appearance of cytoplasmic inclusions, which have been noted by several workers<sup>8,9</sup> but have not as yet been studied in detail, marks an alteration in these volumetric relations. From this point onward, the enlargement of the cytoplasm is of much the same order as that of the nucleus. The progressive change is consequently more marked in the nucleocytoplasmic index than in the nuclear cell index. Before the hypertrophy of nucleus and cytoplasm reach their maxima (about 700 and 2100 cubic microns, respectively) the cells are probably dead — a conclusion which seems justified, on cytological grounds, by the following observations:

In cells only moderately enlarged (100–200 per cent) the cytoplasm when stained by Giemsa's method is distinctly basophilic in contrast to its strong affinity for eosin in the normal secretory duct cells, which points to a swing of the reaction toward the acid side. The mitochondria disappear in accordance with

In both diseases there were many larger cells with pale, round or oval nuclei, which from their frequent relation to blood vessels were probably of endothelial origin. In both diseases there were similar patches of submeningeal infiltration with round cells, the meningitic process extending into these areas in the form of perivascular cuffs, and the cytoplasmic processes of glia cells staining with basic dyes.

In brief, the herpetic meningitis resembles that of neurovaccinia in its essential mononuclear cytology and in its distribution, but it is consistently less severe, the meningeal vessels are not so much dilated, and there are fewer polymorphonuclear cells. Moreover, in the herpetic brains, inclusion bodies may be seen in all types of cells except polymorphonuclears, but they were never seen in the vaccinal cases.

*Blood Vessels:* Vasodilatation was more marked in both meninges and brain substance in the neurovaccinal cases than in the herpetic. In the vaccinal brains an occasional moderate dilatation was seen in such places as the cortex, the base of the midbrain, the corpora quadrigemina and the thalamus. In the cortex, the smallest vessels were the most affected and often contained eosinophilic leucocytes.

Cuffs were seen in all the herpetic brains, and in all except one of the vaccinal. They were more plentiful in the herpetic. They may be divided into two classes:

(1) Submeningeal, *i.e.*, on vessels running in from patches of infiltrated meninges. In "normal" control rabbits the straight vessels perpendicular to the surface are plentifully supplied with nuclei, some endothelial, some of the small round cells. This was a constant finding in the diseased brains, but it was often possible to say definitely that the numbers of both types of cell were increased. Nuclear degeneration (pyknosis) and eosinophiles were not uncommon in the walls of these vessels, with or without a definite increase in the number of cellular elements. These cuffs probably represent direct spread of the meningitic process along the Virchow-Robin space.

(2) Isolated cuffs were seen in various parts of the brain, well removed from the meninges, but it was found impossible to differentiate between such cuffs when they are due to the virus activity, and when they are an expression of spontaneous disease. They were particularly numerous in the one herpetic brain in which the parasite of the spontaneous encephalitis was found.



These scattered cuffs fall roughly into two subdivisions, those that surround the vessel completely, and those that lie to one side of it. The latter kind were rather more common in the vaccinal brains, but both occur in uninoculated brains, including those of four rabbits fourteen days old, which had shown no symptoms of disease during life. In the vaccinal brains small perivascular hemorrhages and occasional perivascular deposits of hyaline fibrinous material suggest a scattered local damage to the walls of the small vessels, an observation which may have significance in view of McIntosh and Scarff's statement <sup>25</sup> that in generalized vaccinia, the lesions are essentially in the vascular endothelium.

Areas of perivascular softening, such as are described in various human encephalitides, were not determined.

*Pigment:* Pigment was noted in only one brain, in the pia mater.

*Edema:* Empty spaces around cells and vessels, and in the fiber tracts were common in all brains.

*Local Lesions:* Injection lesions take the form of areas of hemorrhagic necrosis, containing mononuclear cells and surrounded by diffuse infiltration with eosinophiles and glia cells. In the vaccinal brains no particular changes were noted in the neurones near these lesions, but it is true that in the six "intracerebral" brains what neuron change there was lay further forward in the brain than it did in the two "cysterna" brains. In the herpes brains neighboring changes in the neurones were more marked, and inclusion bodies were commonly found.

Focal collections of cells, whether round cells or glia, occurred in brains of both series, but as similar collections with or without signs of necrosis occurred in five uninoculated control rabbits, no special significance can be attached to them. One such lesion in one of the herpetic brains was accompanied by many inclusion bodies, but this fact alone is not sufficient to prove that the whole lesion was herpetic in origin.

In one vaccinia brain there was a large hemorrhage into the third ventricle, and in another into the external capsule, but both of these were probably caused by the injection injury.

*Changes in Nerve Cells:* There was considerable variation in the amount of change in different brains, but in most of them there was a slight degree of all the changes mentioned below. The herpetic brains showed more change than did the neurovaccinal.

In some of the brains neurone damage was scattered throughout the brain, isolated cells being affected, but in others there were areas in which many of the cells present suffered. Such areas also showed vasodilatation and glial increase.

Unlike the pathological changes in the glia, neuronic changes do not necessarily underlie areas of intense meningitis, nor can their presence or distribution be correlated with the injection injury, save in the broadest way. Changes, other than inclusions, noted in the neurones were:

*Nuclear swelling*, which was the commonest. It was particularly prominent in the herpes brains.

*Chromatolysis*, in all stages, from peripheral condensation of dark Nissl granules to their total disappearance.

*Neuronophagia*, the neurone being in varying stages of disintegration, with the glia nuclei inextricably mixed in its substance. This type of degeneration was rare, but it did occur; Levaditi comments on its absence in neurovaccinia in rabbits. It is not possible to say that its presence in such animals in this series was not due to one of the spontaneous changes already referred to.

*Pseudoneuronophagia*, which, as Da Fano and Ingleby<sup>26</sup> recall, may be distinguished from the true variety by the state of the neurone and the presence of an intact boundary between it and the glia cells.

*Satellitosis*, an increase in the number of satellite cells; an unreliable sign.

*Eosinophilic Degeneration*: The remains of degenerated neurones stain red with eosin. They may be isolated or surrounded by phagocytic microglia. Some nuclei stain, in whole or in part, a purple-red with dyes containing eosin. Their cytoplasm is sometimes shrunken or overstained with methylene blue. This condition is observed in most of the brains and in uninoculated controls. It is to be distinguished from the oxychromatic degeneration of Luger and Lauda,<sup>27</sup> which they describe as the first stage in the formation of herpetic inclusion bodies. The distinction is easily made, for in the Luger and Lauda type, the ground substance of the nucleus stains a deeper pink and has a ground-glass appearance, the chromatin is exaggerated and arranged peripherally, and the cytoplasm is unaffected. Both types are found in the herpetic brains.

*Vacuolation:* Neurones with vacuolated cytoplasm are found mainly in those areas that are badly affected with neuronophagia, etc.

In summarizing these neuronic changes, emphasis must be laid on their infrequent occurrence in the vaccinal brains. The vast majority of the neurones in any one brain show no abnormality.

*Inclusion Bodies:* These constitute the one distinctive feature of the herpetic disease. No inclusion bodies, either intranuclear or cytoplasmic, were seen in any of the vaccinal brains, nor has reference to them been found in the literature. In the herpetic brains they occurred every time. Their appearance coincided with the descriptions of them given by other observers. They were frequently multiple and often accompanied by a fine eosinophilic dust, not unlike that seen in the nuclei of neurones in other conditions; but the "myelin-like" bodies described by Goodpasture<sup>13</sup> were not seen, even though the brains were injected with Zenker's fluid via the carotids while the animal still lived under the anesthetic.

Inclusion bodies were found with considerable constancy in the Ammon horn, where they were accompanied by both types of oxychromatic change and an irregular proliferation of glia cells.

*Neuroglia:* The most marked proliferation of glia occurred beneath the worst patches of meningitis. In these places it was common for the cytoplasmic processes of the cells to stain with methylene blue. In the fiber tracts there was occasional evidence of oligodendroglia proliferation, but this was also seen in some of the uninoculated rabbits. Areas in which there was marked neuronic change also showed some glial increase. Occasionally a glia cell with a small shrunken nucleus and vacuolated cytoplasm, giving an appearance similar to that described by Penfield and Cone<sup>28</sup> as acute swelling of oligodendroglia, was encountered, but the condition was not common.

In the herpetic brains inclusion bodies were not uncommonly seen in glial nuclei. Collections of glia cells into unrelated clumps, with or without signs of necrosis, and subependymal gliosis have already been referred to in connection with the spontaneous lesions. It is difficult to assign any specific meaning to them. Gitterzellen were observed in two brains, in each case just below the acutely inflamed meninges. In material fixed in Zenker's fluid, the vacuoles stained pink and were larger; in formalin sections they were smaller and colorless when stained with Giemsa.

*Changes in the Myelin Sheaths:* Demyelination not unlike that occurring in multiple sclerosis has been noted by Turnbull and McIntosh<sup>2</sup> in postvaccinal encephalitis in man, by McIntosh<sup>3</sup> in encephalitis following smallpox, and by Perdrau<sup>29</sup> in chronic encephalomyelitis in dogs. In these neurovaccinia and herpetic brains a search was made for similar changes by cutting thick frozen sections of formalin-fixed material and staining with Spielmeyer's iron hematoxylin method.<sup>30</sup>

No extensive perivascular, or other destruction of the myelin sheaths at all comparable to that described by the above workers, was found in these acute brains, but scattered diffusely through all the inoculated brains examined were areas in which some of the sheaths were ballooned in a curious and irregular manner. In the majority of the brains these changes were very slight and rare.

Other appearances encountered in these brains as well as in those of the uninoculated controls were small lateral buds on otherwise normal sheaths, an appearance of beading caused by local kinks and bends, occasional small regular dilatations containing small, highly refractile granules, and a few very thin fibers which stained a uniform black.

## DISCUSSION

The findings in this series do not readily lend themselves to any useful generalizations on the pathology of the two diseases studied. The one cardinal point of difference between herpetic meningo-encephalitis and the vaccinal disease is the occurrence of intranuclear inclusion bodies.

Both clinically and histologically the meningitic side of the picture is emphasized in the vaccinal disease, but though greater in extent, it is essentially the same in cytology and distribution as in that of herpes.

Other lesions, perivascular cuffs, scattered neuronc and glial changes, and the mucoid degeneration described, cannot be differentiated from similar lesions of "spontaneous" nature; from this series it is not possible to state that any of them are due to the herpetic or vaccinal viruses alone.

The changes in the myelin sheaths are rare and inconstant, and of obscure significance.

The chronological sequence of events, as judged from these brains and from those of rabbits killed in earlier stages of the two diseases is roughly as follows: The first meningitic change is an infiltration with polymorphonuclear leucocytes, accompanied by some fibrinous exudation. By the fourth day, at which time most of the neurovaccinal animals die, the mononuclear invasion is at its height, but polynuclear cells are still present in considerable numbers. By the sixth day, which represents the time of death of most of the herpetic animals, polynuclears are scarce. Plasma cells are seen occasionally on the third and fourth day, but they become more common at a later date, especially in "chronic" herpes cases, which live to the eleventh or twelfth day. Perivascular cuffs appear early in both diseases, but their origin may be independent of either virus.

#### SUMMARY

1. The histological findings in six neurovaccinal and four herpetic brains are described.

2. Intranuclear inclusion bodies are found to be the only sure distinctive feature of the herpetic disease. In other respects the two diseases are essentially similar.

3. In neurovaccinia, the meningitis is the most conspicuous finding, both clinically and histologically.

4. Various spontaneous lesions in the brains of uninoculated laboratory rabbits are discussed.

5. Myelin sheath changes are described in the brains of herpetic and vaccinal animals, but perivascular demyelination of the kind characteristic of postvaccinal encephalitis in man was not seen. It is possible that the duration of the disease is a factor in the development of such a condition.

Since preparing this paper for publication, valuable articles on the histology of neurovaccinal encephalitis in monkeys and rabbits by Hurst and Fairbrother and by McIntosh and Scarff, have appeared in the *Journal of Pathology and Bacteriology* (1930, 33, 463 and 483). These papers are in agreement on the cardinal points of histology, such as the absence of inclusion bodies and the accentuation of the meningitis. In neurovaccinia, McIntosh and Scarff emphasize the rôle of the vascular endothelium; this is undoubtedly damaged in the smaller vessels and there are occasional evidences of its pro-

liferation, but from the preparations in this investigation the impression is derived that this feature of the disease is subsidiary to the general inflammation, and, moreover, it seems to be just as much a feature of herpetic encephalitis as it is of neurovaccinal.

I wish to thank Dr. Hans Zinsser and Dr. Hugh K. Ward for constant advice and help, Dr. Raymond Morrison for help with many questions of neuropathological import and assistance in staining technique, and Mr. C. V. Seastone, Jr., for the care he has taken with the photomicrographs.

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## DESCRIPTION OF PLATES

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### PLATE 143

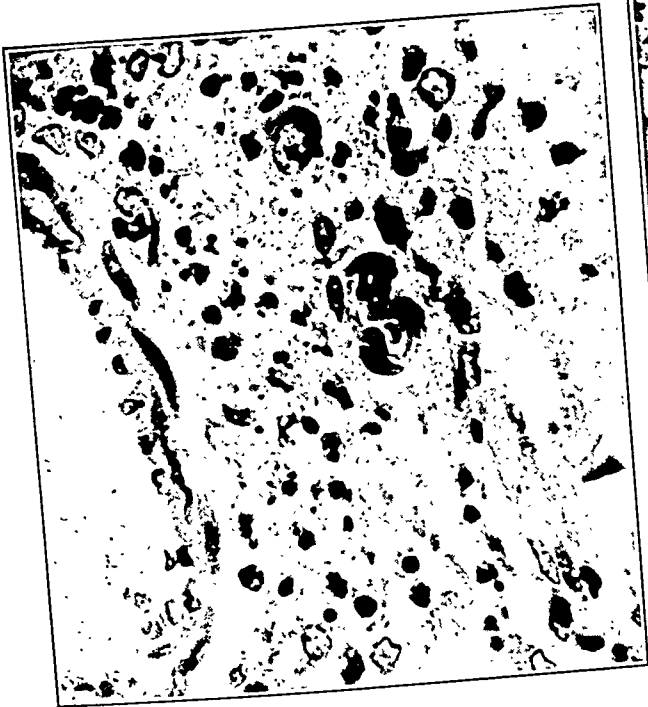
- FIG. 1. Neurovaccinal meningitis in one of the pial septa, showing perivascular arrangement of the mononuclear cells. Formalin fixation. Hematoxylin and eosin stain.  $\times 125$ .
- FIG. 2. Extension of vaccinal meningitis along Virchow-Robin space. Formalin fixation. Hematoxylin and eosin stain.  $\times 125$ .
- FIG. 3. Portion of Fig. 1.  $\times 600$ .
- FIG. 4. Herpetic meningitis. Zenker's fixative. Giemsa stain.  $\times 125$ .



1



2



3



4

Acute Meningo-encephalitis



PLATE 144

FIG. 5. Submeningeal gliosis in neurovaccinal brain. The cytoplasm of the glia cells has stained. Formalin fixation. Giemsa stain.  $\times 600$ .

FIG. 6. "Muroid" change in a vaccinal brain. Formalin fixation. Mallory's phosphotungstic acid hematoxylin and Mayer's mucicarmin stain. (The gray, homogeneous areas stained a bright pink.)  $\times 600$ .

FIG. 7. Neurovaccinia. Vacuolated neurones. Formalin fixation. Giemsa stain.  $\times 600$ .

FIG. 8. Ballooning of myelin sheaths. Spielmeyer's iron hematoxylin method.  $\times 1250$ .

FIG. 9. Ballooning of myelin sheaths.  $\times 600$ .

their almost universal behavior in dead cells. The cytoplasmic inclusions, which will be made the subject of a separate paper, persist. Marked changes occur in the nuclei. The chromatin is greatly reduced in amount and is margined on the inner surface of the nuclear membrane. Thymonucleic acid is reduced to a minimum. Only the nucleolus remains. The hypertrophied cells contain much more potassium both actually and relatively, a fact which seems to indicate that there is a relaxation in the selective properties of the cell membrane. But this breaking up of the internal mechanism which we are accustomed to associate with life is arrested at a certain point. The nuclear membrane, though modified, invariably persists, and it is always easy to distinguish between nucleus and cytoplasm. Yet the cells do not present the usual features of necrosis. They are in some respects like cysts containing much fluid, though the cell membranes are not thickened nor are they folded, but are kept taut, presumably by internal pressure.

It is interesting that the forces which condition the regular course of cellular hypertrophy seem to operate without break over the period of the supposed death of the cells, for the enlargement continues up to the maximum point with but slight alteration in the nuclear cell index. The cells showing the greatest increase in volume maintain a nuclear cell index within the range of gratuitous variability. The absence of extremely large cells from the series makes it clear that there is an end-point in the size increase not far from the mean volume of 2111 cubic microns.

The final result of the hypertrophy does not appear to be the rupture of the cell and the removal of the débris through phagocytic action (or the mechanical effect of the secretory flow) neither is the cell usually swept away in the stream of secretion. On the contrary the affected cells are remarkably resistant and persist seemingly unmodified for long periods — perhaps even throughout the life of the animal. Leucocytic infiltration is conspicuous by its absence except when the virus is injected directly into the gland. Not only do the cells withstand disintegrating forces *in situ*, but, in contrast to dead epidermal cells containing various types of inclusion bodies, they practically never desquamate except in rare cases in the mucous portion of the gland. This failure to desquamate in the serous part, which is the one almost always affected, is the more surprising because the activity of the adjoining cells, oscillations in hydrostatic values and the passage of fluids are factors which one would expect to favor the removal of cells which like these project far into the ducts.

The reason why the inclusions produced in duct cells by the submaxillary virus are larger than any other specific intranuclear inclu-

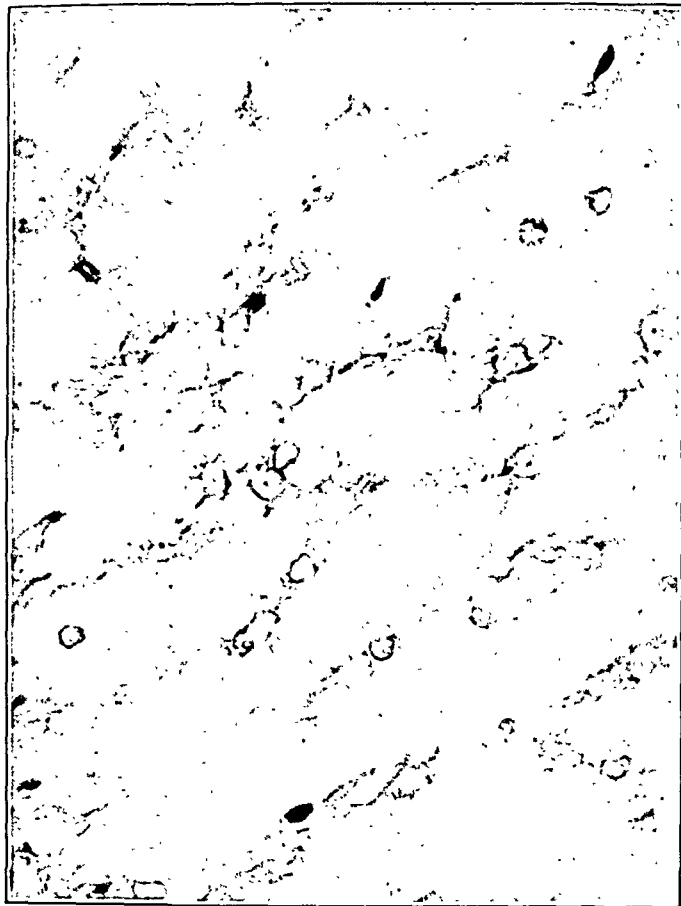
PLATE 145

FIG. 10. Nodular cuff. Vaccinal brain.  $\times 600$ .

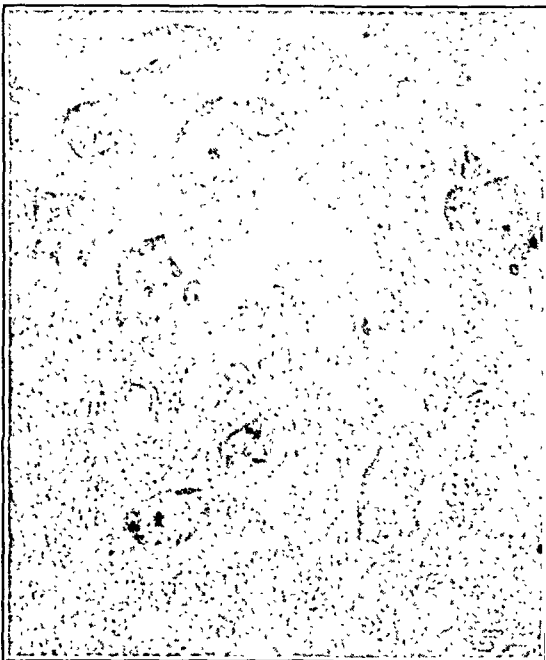
FIG. 11. Lesion containing the parasite of spontaneous encephalitis. Formalin fixation. Carbol fuchsin and methylene blue stain.  $\times 125$ .

FIG. 12. Perivascular lesion in 14 day-old "normal" rabbit. Zenker's fixative. Giemsa stain.  $\times 600$ .

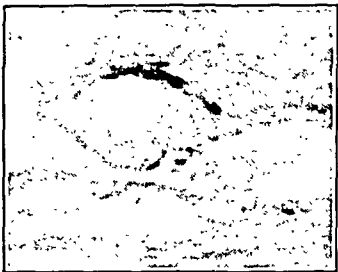
FIG. 13. Parasite of spontaneous encephalitis. Carbol fuchsin stain.  $\times 1250$ .



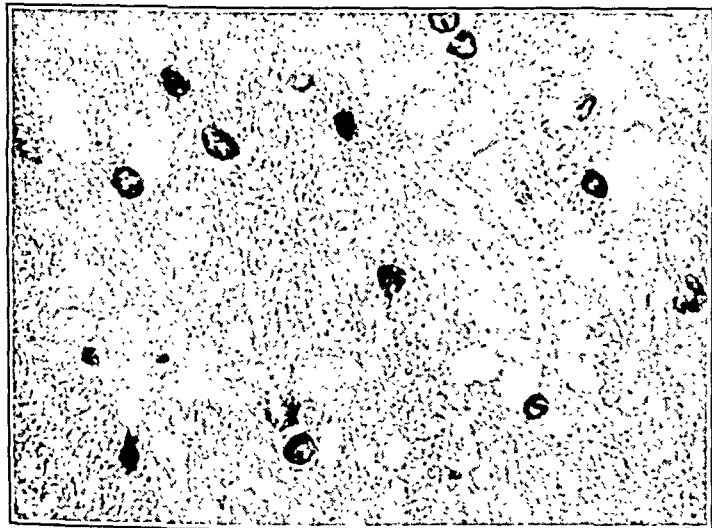
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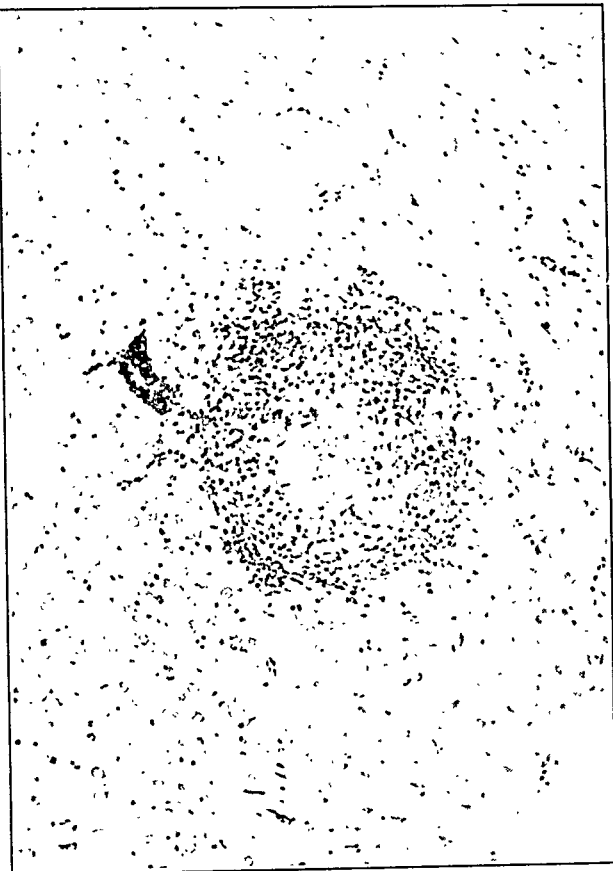


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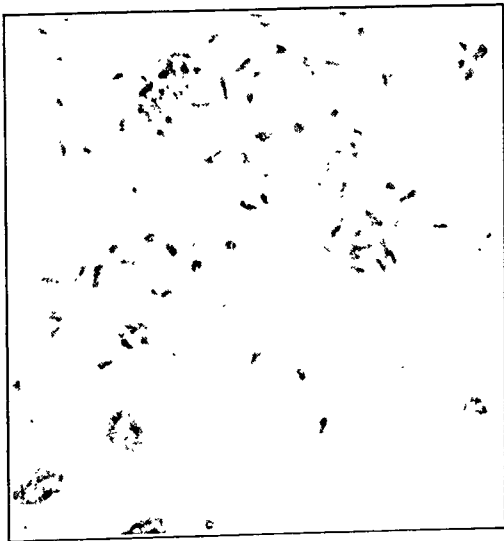
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11



12



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sions in mammals — a fact to which we called attention in the introduction — is not necessarily that the virus acts in any way radically divergent from other viruses though its affinities are of course different. It is rather we think a matter of the kind of cell attacked and its relation to the passage of fluids, for we find that on intracerebral inoculation of the same virus into guinea pigs the nuclear inclusions are not of unusual size, nor do the affected cells undergo anything like a corresponding hypertrophy.

On the exact relation of virus to inclusion body we do not wish to commit ourselves. It may be that the virus is present in the inclusion as the findings of Woodruff and Goodpasture<sup>10</sup> would seem to indicate for fowl-pox; but again we have no evidence. Certainly the bond existing between the submaxillary virus and the affected cells would seem to be one of unusual strength. It is indeed unlikely that any other cells of the body are at regular intervals throughout the life of the animal more thoroughly washed with fluids of low salt content. Their sides and distal surfaces are bathed in water, which on entering the lumina of the ducts is thought to decrease the viscosity of the secretion, which itself by constant passage would tend likewise to dislodge any virus adherent to the proximal ends of the cells.

### SUMMARY

The volume of infected duct cells is greater by 300 per cent than that of the same type of normal unaffected cells. Nuclear volume is increased more than 400 per cent by the presence of the inclusion body. The nuclear cell index is increased from 24.27 to 33.07 during the course of cellular hypertrophy, while the nucleocytoplasmic index is raised from 32.13 to 50.16. The inclusion-nuclear index is practically doubled by the time the cell has reached its stage of maximum hypertrophy.

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## DESCRIPTION OF PLATE

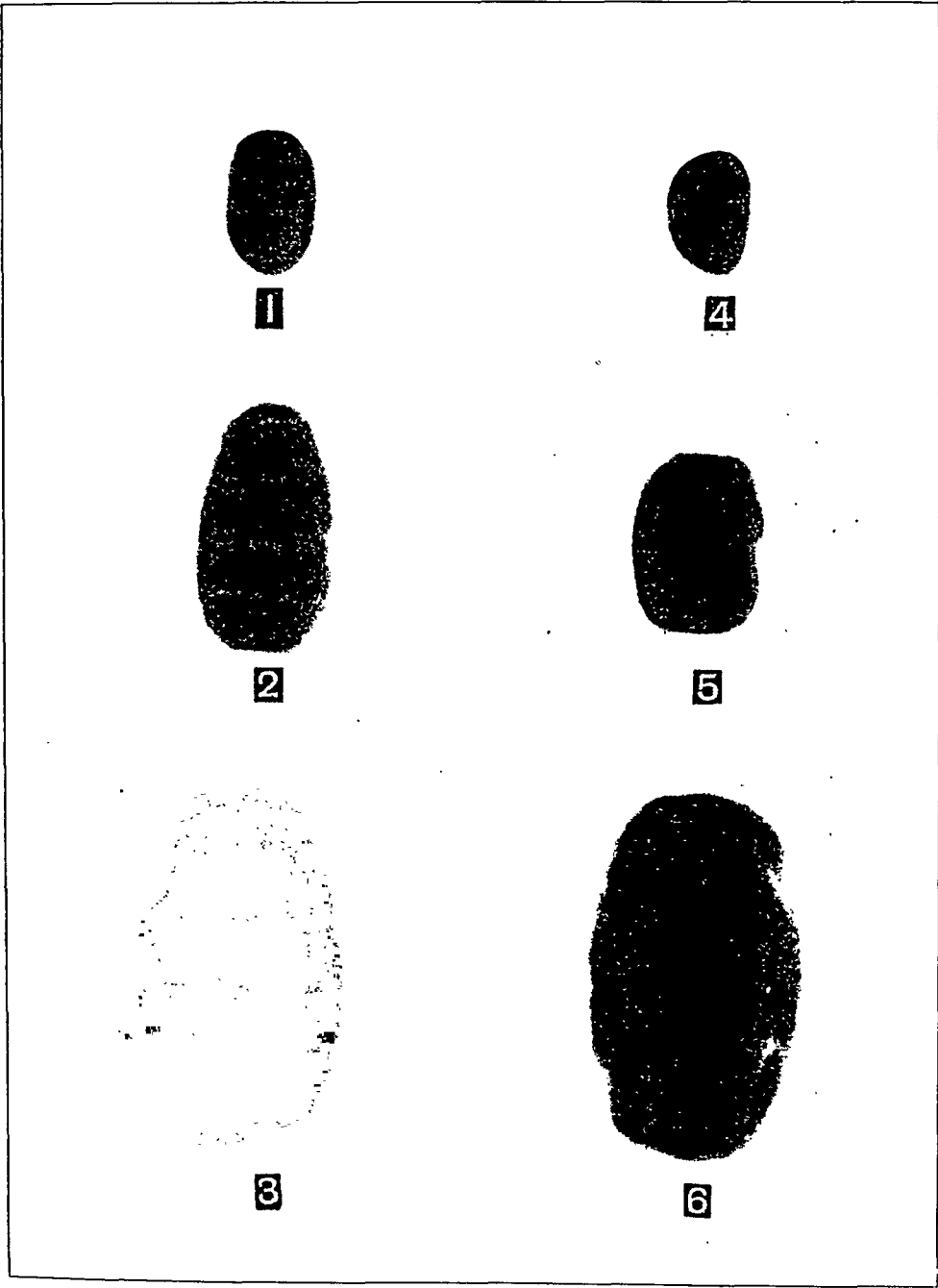
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### PLATE 17

Figure 1 is a photograph of wax models of two cells with late inclusions, each with its nucleus and inclusion body which illustrate their general shape. Each model was built from camera lucida tracings on wax plates of appropriate thickness for a magnification of 1500<sup>3</sup>. The sections from which the cells were drawn were cut at four microns and stained by Giemsa's method. Under these circumstances each cell could be identified in from six to eight sections. 1. Intranuclear inclusion of cell number 1. 2. Nucleus of cell number 1. 3. Cell number 1. 4. Intranuclear inclusion of cell number 2. 5. Nucleus of cell number 2. 6. Cell number 2.







The first four animals in the series which were killed with chloroform immediately following the last feeding of the iodide served as controls. In each case both lobes of the thyroid were cut in serial sections and studied in the manner previously described by us.

TABLE I  
*Number of Mitoses*

Immediately following last KI feeding	One day	Two days	Three days	Seven days	Fourteen days	Twenty-one days	Thirty days
4350	2144	820	200	100	0	0	0
3120	..	..	456	120	0	..	..
1860	..	..	320	..	..	..	..
3250	..	..	..	..	..	..	..
Average 3145	2144	820	325	110	0	0	0

#### NUMBER OF MITOSES IN THE ENTIRE GLAND AT DIFFERENT PERIODS

In Table I we find the individual as well as the average number of mitoses that have been found in the entire thyroid gland of the different animals at the various periods. It will be noted that the number of mitoses in the glands of animals killed immediately after the last iodide feeding is as usual high, averaging 3145 mitoses: this number is in agreement with figures previously obtained under similar experimental conditions. The animal in which the thyroid was removed one day following the last feeding of the iodide still continues to show a considerable proliferation of the acinar epithelium as indicated by the large number of mitoses (2144), a figure which is only slightly below the average. Two days after cessation of KI administration the number of mitoses in the thyroid gland shows a considerable reduction, as indicated by the figure 820 as compared with 3145 in the controls. Three days after cessation of feeding of KI the number of mitoses is still slightly above the average found in normal animals. At seven days it has reached about the normal count or is slightly below the latter, while at still later periods, namely fourteen, twenty-one, and thirty days, the mitotic activity of the epithelium is reduced to zero. It is evident therefore that the effect of KI in

# CHANGES IN THE THYROID GLAND OF THE GUINEA PIG FOLLOWING A PERIOD OF ADMINISTRATION OF POTASSIUM IODIDE \*

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In previous communications <sup>1, 2</sup> reported from this laboratory it has been shown that the oral administration of potassium iodide to normal guinea pigs produces certain characteristic changes in the histology of the thyroid of these animals. These changes are uniform and consist for the most part in a marked mitotic proliferation of the acinar epithelium as well as in a slight increase in size of the epithelial cells and a slight softening of the colloid; at the same time the number of phagocytes invading the colloid increases greatly. Such changes were produced in the thyroid of guinea pigs that were fed daily with from 0.01 to 0.1 gm. KI for a period of two to three weeks, and in which the thyroids were removed from the animals immediately following the last feeding of the iodide.

It was suggested by Dr. Leo Loeb that it would be of interest to determine the length of time necessary for the thyroid to return to its normal state following the cessation of the administration of KI and to investigate the changes which take place in this organ during this period. The following experiments were therefore carried out.

Fifteen male guinea pigs weighing on the average 400 gm. were fed daily with 0.1 gm. KI for a period of fifteen days. The animals were killed and the thyroid removed for examination at different intervals following the last feeding of the iodide according to the following order:

1. Immediately following the last feeding of iodide — four animals.
2. One day later — one animal.
3. Two days later — one animal.
4. Three days later — three animals.
5. Seven days later — two animals.
6. Fourteen days later — two animals.
7. Twenty-one days later — one animal.
8. Thirty days later — one animal.

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two following its last administration. While the number of mitoses in the thyroid is very high immediately following the last dose of KI, this number approaches the normal count within three to seven days later and seems to be reduced to a point below the normal fourteen to thirty days later. Similarly, the other histological changes in the thyroid are most marked in glands that have been removed immediately after the last iodide feeding, and the picture gradually returns to that of a normal gland as more time is allowed to elapse between the last iodide feeding and the removal of the thyroid gland.

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increasing the rate of cell proliferation in the thyroid epithelium rapidly declines after cessation of KI administration. It cannot be prolonged beyond two or three days following the last administration of this substance, or, in other words, the stimulus to cell division produced as a result of KI feeding is effective only when the latter is fed to the animal, but after cessation of the administration of iodide cell proliferation ceases. There is in addition an indication that following the period of stimulation a phase sets in, in which the mitotic activity of the gland is below that of the normal thyroid.

*Microscopic Study:* In the control series, *i. e.*, in animals in which the thyroids were removed immediately following the last iodide feeding, the gland showed the characteristic histological changes that have already been described on previous occasions and have been attributed to KI effects. To recapitulate these changes briefly, they consisted in some softening of the colloid and a marked infiltration of the colloid by phagocytic cells. The epithelium was found to be slightly increased in size. In the case of the animal killed one day following the last iodide treatment, the epithelium was still relatively high, but the colloid was somewhat harder and the phagocytes less numerous than in our control cases. The animal examined after two days showed a very similar picture to that found after one day. In the thyroid removed after three days the colloid exhibited a still greater degree of hardness and showed fewer phagocytes; the epithelium, although slightly taller than that in the normal non-treated cases, was lower than that seen in the control KI animals. After seven days no further change in the structure seemed to have taken place, but glands removed from fourteen to thirty days after the last iodide administration resembled microscopically the normal untreated gland in so far as the colloid was more or less solid, containing only few phagocytes, and the epithelium was low in size. This condition was particularly apparent in those animals in which the gland was removed thirty days following the last iodide feeding.

### CONCLUSIONS

These experiments show that the stimulating effects of KI upon the growth activities of the thyroid gland are manifest approximately only during the time when this substance is being administered to the animal; proliferation begins to decline within a day or

activity of the thyroid epithelium, as evidenced by the high number of mitoses. The number of mitoses in the entire gland of the individual animals varied as follows; 2350, 3112, and 3360, with an average of 2940 mitoses. These figures although somewhat lower than figures previously obtained under similar experimental conditions nevertheless indicate a pronounced increase in cell division. Furthermore, the histological changes in the gland were characteristic of KI stimulation and showed a slight increase in the size of the epithelium, a slight softening of the colloid and a marked increase in the number of phagocytes within the colloid.

2. *Underfed Controls*: The four animals constituting this series were insufficiently fed so that they had lost 11 to 22 per cent of their body weight at the end of the experimental period. The number of mitoses in the thyroid of each of these animals was zero, thus showing a complete inhibition of proliferative activity. In addition, the epithelium was lower than normal and the colloid was harder and very scantily infiltrated with phagocytes. The histological changes of the thyroid gland in these cases indicated, therefore, a very much diminished activity: it was very similar to that described in the previous paper by Rabinovitch.

3. *Underfed Guinea Pigs Treated with KI*: In this series the animals lost 17 to 26 per cent of their body weight because of undernutrition; the number of mitoses in individual cases was 60, 0, 50, 0, 0, 0. These figures approach, therefore, very closely those obtained in the underfed animals not treated with potassium iodide. Evidently the under-nourishment and subsequent loss of weight of the guinea pigs is very injurious to the activity of the gland and counterbalances the stimulating effects of the iodide. There does not seem to be, however, a complete parallelism between the degree of loss of weight and the resulting diminution in cell division, for some of the animals that suffered the greatest loss of weight (26 per cent) had comparatively more mitoses (50) than the animals which lost least weight (17 per cent); some of the latter had no mitoses at all. However, the effect of underfeeding was present in all cases and the differences found between different individuals were relatively slight. In addition to the diminution in cell proliferation, the epithelium was found low cuboidal and the colloid mostly solid with only occasional areas of softening, while the phagocytes were diminished in number and observed only in the softened areas.

# THE EFFECT OF POTASSIUM IODIDE UPON THE THYROID GLAND OF UNDERFED GUINEA PIGS \*

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Loeb <sup>1</sup> has emphasized the importance of changes in weight in his work on compensatory hypertrophy of the thyroid gland. He noticed that compensatory hypertrophy and the effect of potassium iodide upon this process were diminished in animals that had lost weight. Rabinovitch <sup>2</sup> subsequently showed that the mitotic cell proliferation which occurs normally in the thyroid gland of guinea pigs can be completely inhibited by underfeeding. In continuation of these experiments on the thyroid gland, it was thought of value to study the effect of underfeeding upon the thyroid glands of guinea pigs who had been fed potassium iodide. These glands would normally show very marked proliferation <sup>3</sup> of the acinar epithelium, an increase in the size of the epithelium, a softening of the colloid and an increase in phagocytes. Under these conditions, if underfeeding had an inhibitory effect, that effect would be more pronounced. This report is the result of a quantitative study of this subject.

Thirteen male guinea pigs averaging in weight between 400 and 450 gm. were divided into the following three groups: (1) normal guinea pigs fed with KI, three animals; (2) underfed guinea pigs, four animals; and (3) underfed guinea pigs treated with KI, six animals. The experiments were extended over a period of fifteen days during which time the underfed animals lost between 11 and 26 per cent of their original body weight, while the normal animals gained between 10 and 24 per cent. At the end of this period the animals were killed with chloroform and the thyroids removed and studied in a manner already described in previous communications from our laboratory.

1. *Normal Controls Fed with KI:* The gain in weight observed in these animals varied between 10 and 24 per cent during the course of the experiment. We found in these cases a marked proliferative

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## DISCUSSION

These experiments illustrate that undernutrition plays an important rôle in the activity of the thyroid gland. The manner in which the undernutrition affects the activity of this gland is rather problematic; it may be assumed however, that the diminished food intake deprives the thyroid of a certain amount of its required nutrition, without which it cannot perform its normal function. Under such conditions KI, which normally stimulates the thyroid epithelium toward increased cell division, fails to be effective and the gland assumes a quiescent resting stage showing a low epithelium and solid colloid. Such a histological picture we find to be characteristic of an inactive gland. These experiments add therefore additional proof to our previous conclusion, that the maintenance of body weight of the animal is an important factor in regulating the activities of the thyroid gland. Our experiments furthermore emphasize again the necessity of considering changes in weight taking place in guinea pigs during the experimental period in estimating the effects of various factors on the growth and function of the thyroid gland.

## SUMMARY

The underfeeding of guinea pigs to which potassium iodide is being administered results in suppression of cell proliferation of the thyroid gland.

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capsule. But a search through the literature reveals only twenty cases of a diffuse involvement of the spleen such as reported here. The diagnosis was usually made only microscopically; in most cases the spleen was described grossly as having the appearance of a congested or amyloid spleen: Sappington's case showed "granite-like mottling." These cases are collected here for reference.

Di Biasi found secondary growths in the spleen in fifty of 2422 autopsies on carcinoma (2 per cent); in one of these there was a diffuse involvement with a microscopic picture closely resembling that in the case reported here. Chalатов reports six cases of carcinomatous metastases to the spleen and in all of them one or more sharply outlined nodules were seen. In one the cut surface was strewn with nodules varying in size from that of a pin-head to that of a cherry-stone; in another, besides two large macroscopic metastases, small groups of cancer cells were seen in all parts of the spleen and in the vessels. Geipel reports five cases of diffuse involvement of the spleen, two of which he lists as secondary to "sarcoma" of the bronchus: it seems possible that these two were carcinoma rather than sarcoma and they are therefore included in this series. Two of Kettle's <sup>8</sup> four cases of metastases to the spleen showed a diffuse infiltration with carcinomatous cells. Kraft <sup>9</sup> reports the interesting case of a woman of 49 years who had had pallor and weakness for seven months; she was found to have a lump in the left breast and enlarged axillary glands. A radical amputation of the breast was done and the operative specimen was reported as scirrhus carcinoma. There was no improvement in the symptoms following operation and two months later the spleen, weighing 2000 gm. was removed. The other viscera appeared normal. Microscopically, the spleen was diffusely infiltrated with what was diagnosed as scirrhus carcinoma. The patient improved and was living and well four months after the second operation. Lyter <sup>10</sup> has a somewhat similar case; a carcinomatous breast was removed, following which fifteen X-ray treatments were given; weakness, cachexia and anemia developed and the patient died eight months after the operation. At autopsy the spleen and bone marrow were diffusely involved by metastases. Von Parsch <sup>11</sup> reports seven cases of diffuse carcinomatous metastases in the spleen; unfortunately his report is very brief and omits details. Sappington's case, one of carcinoma of the breast, was in several respects similar to ours. Clinically, electric

# METASTATIC CARCINOMA IN THE SPLEEN \*

## REPORT OF A CASE

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Metastasis of carcinoma to the spleen has always been recognized as a rare occurrence. Rokitsansky in his text-book of 1855 says that it occurs, and mentions having seen an instance twenty years before. Beginning with Gussenbauer and von Winiwarter<sup>1</sup> in 1876, the incidence of metastasis to the spleen in a large series of cases has been reported from time to time. The literature has been reviewed more or less fully by di Biasi,<sup>2</sup> Chalataw,<sup>3</sup> Geipel,<sup>4</sup> Sappington<sup>5</sup> and Yokohata.<sup>6</sup> Yokohata recently collected the figures from twelve series of cases and found splenic metastases reported in 313 of 17,783 autopsies on carcinoma, an incidence of 1.76 per cent. The figures from individual series vary from those of Paget and of Reichelmann (2.3 per cent and 2.4 per cent respectively) to those of Handley, who found only one splenic metastasis in 422 cases (0.25 per cent). The form of the metastases varies widely from single or multiple macroscopically visible nodules sharply demarcated from the surrounding splenic tissue, or even encapsulated by connective tissue, to diffuse growths infiltrating the spleen and calling forth little or no visible connective tissue response. It is to this latter very unusual form that we wish to call particular attention in this paper. Yokohata believes that the frequency with which microscopic metastases are found varies with the care exercised in looking for them. He studied from five to twenty microscopic sections of the spleen in each of twenty-nine consecutive cases of carcinoma coming to autopsy and found microscopic metastases in ten cases, or 34 per cent. He cites the similar work of Deelman<sup>7</sup> who found microscopic metastases in seven of seventy-five cases in which no gross nodules were seen in the spleen.

By far the most frequent form of the metastases is the isolated nodule, which may or may not be surrounded by a definite fibrous

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## MICROSCOPIC EXAMINATION

Microscopically, the main tumor mass is found to consist largely of dense fibrous tissue, invaded by large cells with large vesicular nuclei, sometimes singly, sometimes in strands and sometimes in groups resembling acini. In the adjacent lymph nodes the relative amount of connective tissue is considerably less, while in the visceral metastases the connective tissue is scant. The metastases are extremely diffuse, groups of cells being found in the connective tissue surrounding bronchioles and in the lymphatics of the lung, in the myocardium, around the central veins of the liver, in the interlobular connective tissue of the pancreas, in the wall of the urinary bladder, and replacing the bone marrow. Attached to the cortex of the spleen are several fibrous tags, in many of which strands of cells similar to those seen in the breast are found. The trabeculae are well marked. The malpighian corpuscles are small and scattered but the cells do not appear unusual. There are a few small round cells in the pulp but most of the tissue is made up of neoplastic cells similar to those found in the breast. They are so diffusely scattered throughout all the sections studied, in small clusters or occasionally in structures resembling alveoli, that the tissue is almost unrecognizable as spleen. An occasional group of such cells is seen in a blood vessel. A Mallory anilin blue stain shows only a very delicate connective tissue framework supporting the neoplastic cells.

The extremely widespread metastases in this case and in that of Sappington's may very well be associated with the ill-advised treatment given, massage in one and the application of a salve, probably also by massage, in the other. The anemia is probably due in part, at least, to involvement of the bone marrow, as in Lyter's case.

## DISCUSSION

The pathway by which the spleen is invaded is discussed by di Biasi, Geipel, Sappington, and others. Of course direct extension from a neighboring viscus occurs, but is not to be considered as metastasis. Di Biasi analyzes the pathway in his fifty cases as follows: by direct bloodstream extension, seventeen cases; probably, but not certainly, by the bloodstream, fourteen cases; by lymphatic or direct extension, sixteen cases; uncertain, three cases: that is to say,

treatment and massage had been used; postmortem examination showed diffuse secondary involvement of practically all of the organs, including the spleen. In addition to these cases Marschoff<sup>12</sup> cites Biermer's case with diffuse infiltration of the spleen, the primary source being a carcinoma of the liver.

### REPORT OF CASE

*Clinical History:* A colored laundress, aged 65 years, was admitted to the New Haven Hospital with the history and physical findings of congestive heart failure. In addition to this, three years before admission she had noticed a painless lump the size of a hen's egg in her left breast; a doctor diagnosed it as a "wash-woman's tumor" and prescribed some salve, which she continued to use until the time of her admission. She lost 15 pounds in weight during the four years preceding admission. Physical examination revealed an aged, emaciated colored woman, breathing rapidly and with obvious difficulty. The physical findings of interest here were a firm nodular mass in the left breast, adherent to the skin but not to the underlying ribs, and large hard lymph nodes in both axillae, in the right breast and in the supraclavicular fossae. The skin over the left breast was wrinkled but was not ulcerated. There was pitting edema about the left elbow, attributed to pressure of the enlarged glands on the venous circulation. The rest of the physical examination showed the signs of circulatory failure. The red blood cell count was 1,700,000, the hemoglobin 30 per cent. There was no improvement in the patient's condition, but rather a progressive downhill course to death on the tenth day, with signs of congestive heart failure.

### AUTOPSY

The findings on external examination are essentially the same as the clinical findings, except that the edema about the left elbow has disappeared. The mass in the left breast measures 3 by 6 cm.; it begins beneath the nipple and extends obliquely upward and outward, becoming almost continuous with the enlarged, hard confluent nodes in the axilla. This mass cuts with great resistance; it appears grossly to be made up of dense white fibrous tissue, in which there are numerous opaque yellow streaks. The nodes in the axillae and in the right breast are similar in appearance. On opening the abdomen the peritoneal surfaces are found to be studded with small metastatic nodules. Similar nodules are found in the substance of the liver, the kidneys and the adrenals, in the visceral and parietal pleurae and in the pericardium. The spleen weighs 110 gm. and measures 10 by 6.5 by 3.5 cm. It is dark red and firm. The capsule has numerous fibrous tags which bind it to surrounding structures. On section the malpighian corpuscles can be seen and the trabeculae stand out prominently.

The view that the spleen's immunity is to be explained on a mechanical basis does not lend itself readily to experimental proof or disproof. On the other hand, a great deal of work has been done to show that there is, or is not, something inherent in the spleen which makes it unsuitable soil for neoplastic growths. The volume of this work is so great and the results so conflicting and inconclusive that it does not seem worth while to try to cite it in detail.

Tumors which regress either spontaneously or under radiation therapy show an increase in lymphocytes in the surrounding tissue. This observation has led to the opinion that lymphoid tissue exerts a deterrent effect on neoplasms. Attempts to show an increased susceptibility to transplanted carcinoma following reduction of the lymphoid tissue by X-ray, has in some experiments yielded positive, in others negative, results. Similarly lymphocytosis has in some experiments apparently been accompanied by an increased susceptibility; in others no change was observed. Since the spleen is the most convenient source of lymphoid tissue it has been most used for experimental purposes. Many experiments have been reported to show that the growth of transplanted neoplasms is more rapid in splenectomized than in normal animals; that it is retarded in animals simultaneously injected with spleen extract; and that it is still more retarded by extracts of the spleens of animals already affected with cancer. Other experiments by equally qualified investigators, using similar technique and, in some cases the same strain of transplantable tumor, have shown that none of these procedures has any effect whatever on the rate of growth of the tumors. Evidence has been brought forward to show that antibodies are formed in the spleen, lymph nodes, and bone marrow which can be used therapeutically or prophylactically in experimental animals; these antibodies have not been demonstrated by *in vitro* experiments such as the precipitin or complement-fixation reactions. Some investigators have thought that ferments in the spleen destroy the implanted cells. Spleen emulsion has been tried therapeutically on carcinoma in human beings, but without effect. Lieblein <sup>13</sup> incubated spleen extract with carcinoma cells and observed no cytolytic effect on the cancer cells. According to Goldman <sup>14</sup> cancer transplanted directly into the spleen grows as readily as anywhere else in the body. This in itself seems to be a most convincing argument against any specific immunity of the spleen.

in 31, or 62 per cent, of the cases, the splenic metastases were probably due to bloodstream dissemination. In one of Geipel's cases, with the primary seat in the pancreas, the portal vein and several of its tributaries, including the pancreatic and lienal veins, were filled with carcinoma cells; these cells were seen invading the walls of veins in the spleen, but were not to be seen in the capillaries. He therefore believes that the lienal vein was the pathway of extension in this case, and also that retrograde extension through the portal vein from a primary or secondary growth in the liver must be considered in other cases of carcinomatous metastases to the spleen. Von Parsch finds in some of his cases that the large veins are filled with tumor cells while the splenic tissue is unaltered, and offers the same explanation as Geipel. Sappington attributes the splenic metastases in his case to bloodstream dissemination from the secondary growths in the lungs; we believe that to be the pathway in the present case.

The relative immunity of the spleen to successful implantation of metastatic growths has occasioned much discussion and speculation and considerable experimental work. The explanations offered can be divided roughly into two classes; those based on the reaction of the tissue to implanted cells, and those based on mechanical considerations. In the former view, upheld by Chalatow and Kraft, ferments and hormones or the phagocytic cells of the spleen are believed to destroy the invading cells. A mechanical explanation, according to Sappington, can be based on three factors: (1) there are no afferent lymphatics; (2) because of the sharp branching of the splenic artery from the celiac axis the large metastatic cells are carried past and do not enter it; and (3) the rhythmic pulsation of the spleen keeps the tumor cells oscillating and prevents their getting a foot-hold. Of these three the first seems the most reasonable and is in agreement with anatomical facts. As for the second, the sharp branching of the splenic artery does not prevent the spleen from being one of the most frequent sites of infarction, caused by emboli presumably much larger than the tumor emboli. And as for the third, the rate and amplitude of motion in the lungs is much greater than in the spleen, and yet the lungs have frequent metastases. It must be remembered, however, that all the blood of the body passes through the lungs at each cycle, while only a small part passes through the spleen.



In view of these contradictory results it is not surprising that in recent years this line of investigation has been almost entirely given up. The question of the immunity of the spleen in particular and of the body in general is unsettled; undoubtedly the multiplicity of explanations is due to the same cause as the multiplicity of drugs used in the treatment of certain diseases; namely, the fact that none is found very satisfactory.

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carried out. In this paper we wish to report a series of experiments with gases in which a hitherto unstudied phase of their effect within the body will be described. This phase is the local tissue reaction to subcutaneous injections of pure gases.

In our experiments small amounts of sterile oxygen, nitrogen, and carbon dioxide were introduced into the subcutaneous tissues of guinea pigs and the reactions of cells and tissues to the individual gases were studied. As far as we know this is the first time that such studies have been made. Gases have been introduced into the subcutaneous tissues under other conditions, but the object of the injections has been for some other purpose, and the local inflammatory reaction has been disregarded. Boeldieu <sup>1,2</sup> and others have injected nascent oxygen subcutaneously in the treatment of whooping cough, and various devices for the introduction of this gas into the tissues have been perfected, notably by Fialip <sup>3</sup> and Agasse-Lafont and Douris.<sup>4</sup> Oxygen has also been introduced therapeutically into tuberculous abscesses with good results. Bonnamour and Langénieux <sup>5</sup> have studied the effect of subcutaneous injections of carbon dioxide on the respiration. Campbell <sup>6</sup> introduced various gases, especially oxygen, nitrogen, and carbon dioxide, into the subcutaneous tissues and studied the diffusion of gases from the blood. He found that after several hours the gas originally injected was no longer pure, but contained, mixed with it, other gases which had come by diffusion from the blood. McIver, Redfield, and Benedict <sup>7</sup> obtained similar results when the same gases were injected into isolated loops of intestine. But nowhere in the literature concerning the injection of gases into the animal organism have we found any mention of the study of the tissue response at the locus of injection. Of interest, however, is the brief description of the histological appearance of emphysematous mediastinal tissues given by Wolbach <sup>8</sup> in his discussion of the pathology of fatal influenza cases studied at an army camp during the epidemic of 1918. Here the gas, atmospheric air, reached the mediastinal tissues from the lungs, and Wolbach calls attention to certain histological changes which he observed in the emphysematous regions. In the present paper we wish to limit ourselves to the local subcutaneous reaction and to describe it in some detail.

These experiments have been carried on in close association with the group of studies on the tissue reactions in tuberculosis being

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## THE LOCAL EFFECT OF THE INJECTION OF GASES INTO THE SUBCUTANEOUS TISSUES\*

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The introduction of various gases into the animal organism for purposes of experimental study or for therapeutic effect has been carried out for many years. The types of gases which have been used have varied greatly. Oxygen, nitrogen, carbon dioxide, hydrogen, atmospheric air, and mixtures of some of these, have all been employed. The methods by which these gases or gaseous mixtures have been introduced have likewise varied. In some cases the experimental animal has been enclosed within a chamber in which the gas content could be accurately regulated. In others the gases have been supplied by means of a mask or nose-piece through which a known quantity or type of gas could be delivered. Again the gas has been actually injected into the body, either into a natural cavity such as the pleural cavity, or the lumen of an isolated loop of intestine; or directly into the tissue spaces. Evacuated abscess cavities have been filled with certain gases (*e. g.* oxygen), and on rare occasions gases have been introduced into the blood stream.

It is not our purpose to enter here into a discussion of these many and unrelated studies made with the gases mentioned above. It is desired simply to indicate at the outset that such studies have been

\* The major part of this work was done in the Department of Pathology of the Vanderbilt University Medical School. All of it was throughout in close association with, and represented an integral part of the program of work that is being carried on in the Department of Anatomy with the help and coöperation of the Research Committee of the National Tuberculosis Association.

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In 1928, Lawrence, Tompkins and Cunningham,<sup>14</sup> using yellow phosphorus dissolved in various oils, were able to bring about the production of large numbers of monocytes and epithelioid cells by the injection of this substance into the subcutaneous tissues of guinea pigs. It was thus shown for the first time that certain chemical irritants, in addition to the tubercle bacillus, could produce these cells.

The problem then was to determine the character of the various stimuli which would bring about an increase in monocytes and the production of the epithelioid type of cell. Was the reaction one in which the irritating agents were similar to each other chemically? Or was the cause purely physical and non-specific, unrelated to the chemical structure of the agent? In order to throw some light on these questions, it was decided to carry out experiments employing the simplest, most elemental irritants, and the gases named above were selected.

*Technique of Injections:* The subcutaneous injections of gas were given with a very simple mechanism which consisted of a 20 cc. syringe to the outlet of which was attached a three-way valve which permitted the connection not only of the needle but also of a side-tube through which gas could be drawn into the syringe from the gas container. Gas was conveyed to the syringe through a sterile rubber tube, pressure tubing being constantly employed. Before entering the syringe, however, the gas passed through sterile dextrose broth contained in two test-tubes which were inserted between the gas chamber and the syringe. Following the injections into the animals these culture tubes were removed and incubated at 37.5°C. At the end of twenty-four and forty-eight hours, the broth was examined for evidence of bacterial growth. This procedure was carried out for a considerable period of time, and in not a single instance was there any growth in the medium. It was then decided to dispense with the culture tubes and to replace them with a small sterile glass tube partly plugged with sterile cotton. This worked excellently and in no case was there evidence of local infection in the tissues which had been injected with the gases.

The gases used were obtained from commercial sources, coming in large steel tanks in which they were held under great pressure. By means of a reducing valve, the flow of the gases could be so diminished that it was possible to remove small amounts at a time.

carried out at the Vanderbilt University Medical School. It therefore seems worth while, at the outset, to outline briefly the major contributions to this subject and to indicate the connection which these seemingly unrelated studies with gases have to the study of the tissue reactions in tuberculosis.

In the early study of the epithelioid cell of the tubercle, Baumgarten is the outstanding figure. In 1885 he reported his classical observations on the tubercle as studied in the avascular cornea of the rabbit.<sup>9</sup> He called attention to the "epithelioid" character of the typical cells of the lesions, the presence of which forms the essential criterion upon which the histological diagnosis of tubercle rests. As Krause<sup>10</sup> has pertinently remarked: "Epithelioid cells, onion-layered, may alone be seen, as tubercle bacilli have disintegrated and disappeared; it is a tubercle. Tubercle bacilli may alone be present, it is not a tubercle, nor is it one if lymphocytes alone display themselves, or a giant cell alone."

In 1923, Sabin (quoted by Cunningham<sup>11</sup>), in studying the scrapings of a lymph node from a patient with tuberculosis first observed these typical cells of the tubercle while living, and noted their similarity to the monocytes of the blood. Stimulated by her findings, Cunningham, Sabin, Sugiyama and Kindwall<sup>12</sup> systematically studied experimental tuberculous lesions in the rabbit. They limited their descriptions to observations made on supravitaly stained preparations, and their results confirmed Sabin's earlier findings. They found that monocytes and epithelioid cells were present in great numbers in all tuberculous tissue and that transitional stages from one type to the other could be demonstrated. They introduced criteria for the identification of the individual epithelioid cell which up to then could not be recognized apart from its presence in a tubercle. In their report the typical epithelioid cell was described and pictured and the steps in its transition from the monocyte were followed.

From their studies they concluded that the tubercle bacillus, from an intracellular position, in some unknown way, brought about the change of the monocyte into the epithelioid cell. Somewhat later, however, Sabin and Doan,<sup>13</sup> working with chemical fractions of tubercle bacilli were able to produce typical epithelioid cells in rabbits with the phosphatid fractions, A-3 and A-4.

Zenker's fluid in order to fix the films so that they might be stained later by other methods.

After the material for the supravital studies had been obtained, all of the subcutaneous tissues of the injected area were excised and fixed in Zenker's fluid. They were later embedded in paraffin and sectioned. Such sections were then stained by various methods, the usual being the ordinary hematoxylin-eosin method. Many sections were also stained for fibrin and collagen with Mallory's anilin blue and Mallory's phosphotungstic acid hematoxylin stains. In a number of cases, sections were stained for fibrin by Weigert's method and for elastin by Verhoeff's method.

*Rate of Absorption of the Gases:* Although not considered at first to be an integral part of the principal problem, the period of time necessary for the complete disappearance of the gases from the subcutaneous tissues assumed a more and more important aspect as distinct differences in their absorption became evident. Carbon dioxide, for example, disappeared from the tissues readily. Within six to eight hours after the injection of 20 cc. of this gas only the slightest trace remained. If double the amount, 40 cc., were injected crepitation could be demonstrated for about twenty to twenty-four hours. This evidence of rapid absorption of carbon dioxide agrees with that of other workers who attribute the rapid disappearance of the gas to its very high diffusion rate in tissue. Campbell, for example, found that carbon dioxide disappeared from the subcutaneous tissues of rabbits very rapidly. McIver, Redfield and Benedict found the same to be true of carbon dioxide injected into an isolated loop of intestine.

Nitrogen, on the other hand, remained for long periods of time in the tissues. At the end of forty-eight or even seventy-two hours, the gas was still present in demonstrable quantities. This agrees with the findings of other observers, especially those already mentioned. When 20 cc. of this gas were introduced into the subcutaneous tissues crepitation was observed for a three-day period. Rarely were injections of nitrogen given at more frequent intervals.

In the rate at which it disappeared from the tissues, oxygen occupied a position approximately midway between the other two gases, perhaps more nearly approaching carbon dioxide than nitrogen. At the end of twenty-four hours after a 20 cc. injection, slight crepitation was usually demonstrable and small bubbles of gas

The individual gases were not analyzed or purified chemically, but were used directly from the tanks.

Before each experiment, the syringe, valve, needles and the rubber and glass tubing were sterilized by boiling. When cool they were attached to the gas chamber, and gas was run through the system long enough to assure a supply of the pure gas into the syringe. The needle was then carefully introduced into the subcutaneous tissues of the animal and approximately 20 to 25 cc. of the gas were injected. In the case of carbon dioxide, it was found necessary to introduce 40 cc. or more on account of the rapidity with which this gas was absorbed by the tissues. The animals were kept in clean cages until the following day when the injections were repeated. This procedure was carried out daily for varying lengths of time, although it was soon found that nitrogen disappeared so slowly that injections of this gas were necessary only at three or four-day intervals. At various periods, the animals were sacrificed and the tissues studied.

*Methods of Study:* Immediately after the death of the animal the tissues and cells of the exudate were studied in the living state by means of the supravital technique.<sup>15</sup> The slides employed were coated with a fairly strong solution of neutral red alone (30 to 60 drops of the concentrated stain in 10 cc. of absolute alcohol) or with a combination of neutral red and Janus green, 2 drops of a saturated absolute alcoholic solution of the latter dye to 1 cc. of the diluted neutral red. Small amounts of the exudative fluid were scraped from the subcutaneous tissue, placed on clean cover slips, dropped on a slide, and sealed. In addition, minute fragments of connective tissue were spread out as thinly as possible on cover slips. During manipulation, this tissue often became slightly dry. To replace the evaporated fluid, a small drop of sterile serum from a normal guinea pig was added to the tissue before it was placed on the slide. All of the microscopic examinations of these fresh preparations were made in a hot box which was kept at a constant temperature of 39°C.

Studies were made within an hour after the spreads were prepared. Specimens more than an hour old were discarded because of the rapidity with which cell degeneration took place after that time. When the supravital preparations had served their usefulness the cover slips of many of them were carefully removed and dropped in

## SUPRAVITAL STUDIES

*Early Reaction:* The local reaction at the end of short periods of time, twenty-four to forty-eight hours, was quite different from that occurring at later stages, after four days, for example. The acute reaction with all gases tended to be a granulocytic one, although monocytes in varying numbers, from 30 to 60 per cent, were also present. With oxygen, polymorphonuclear neutrophilic leukocytes were quite numerous, sometimes reaching 60 to 70 per cent of the total cells in preparations made at the end of twenty-four hours, and somewhat less at forty-eight hours. Nitrogen produced a definite, though less marked, granulocytic reaction at the end of twenty-four hours, about 40 or 50 per cent of the cells being of this type. At forty-eight hours, the granulocytes were much less common and monocytes were the predominant cells. Carbon dioxide at both twenty-four and forty-eight hours produced but a slight reaction. At that time polymorphonuclear neutrophils were the predominating cells.

*Late Reactions. Smears:* In all preparations, early as well as late, there were varying numbers of moderately large cells, measuring from 8 to 12 microns in diameter. These were round or oval in shape, usually with smooth, regular outlines, occasionally irregular in shape and containing curved, oval or indented, eccentric nuclei which were clearly visible. Within the cytoplasm and grouped usually in a rounded, rosette-like clump near the nucleus there were varying numbers of light brownish red or yellowish red vacuoles. Sometimes these surrounded a clear zone, the centrosphere, but more often they were grouped in a single mass, the center of which was less dense than the periphery. In many cells they were not so grouped, being found above or below the nucleus or scattered throughout the cytoplasm. They varied but slightly in size. Most of them were smaller than similar bodies in the normal monocyte of the blood. In addition to the neutral red vacuoles there were occasional refractile droplets of fat.

These cells were present in all preparations examined. They were found even after twenty-four hours, but appeared in considerable quantities at the end of forty-eight hours and were most numerous at four to six days or thereafter. Many of them resembled the typical monocyte of the circulating blood, but the majority showed some

could be found in the corium on gross examination. After thirty-six hours, however, the gas had usually left the tissues completely.

### OBSERVATIONS

*Gross Description:* Before the animal was killed it was nearly always possible to demonstrate by crepitation the presence of free gas in the tissues. With carbon dioxide this was a less common finding on account of its rapid absorption, but with oxygen and nitrogen there was usually a considerable amount of gas present. When the animal was killed and the subcutaneous tissues were exposed, they were found to be quite moist or even wet. The wetness varied somewhat with the different gases, being most marked with nitrogen and least with carbon dioxide. The maximum amount of fluid was present at four to ten days, sometimes later. In the case of nitrogen, the tissues were often so filled with moisture that large amounts of fluid, often filled with macroscopic vacuoles of gas, could be scraped up with the knife.

Injections of the local vessels varied in intensity, sometimes being quite marked, but more commonly appearing only in local regions near the sites of needle punctures.

The exudative fluid, when abundant enough to examine closely, was grayish white in color and not infrequently tinged with blood from vessels which were torn during the process of scraping. Usually bubbles or vacuoles of gas were visible to the naked eye throughout the tissues, being most numerous when nitrogen had been injected, and only slightly less so following oxygen administration. In the carbon dioxide animals, however, visible gas vacuoles were never present in great number, and often no such vacuoles were seen. The gas vacuoles were in all cases more numerous in the soft tissues of the groin than they were in the subcutaneous connective tissue of the anterior abdominal wall.

At early periods, up to five or six days, there was no recognizable thickening of the subcutaneous connective tissue, but at later stages, *i. e.* after ten days, there was a moderate increase of the fibrous tissue with resulting induration and firmness of the abdominal wall. This was most noticeable in the case of nitrogen, and least remarkable with carbon dioxide.

animals. With carbon dioxide they were less common at all stages. These cells were usually very large, fifteen to forty microns in greatest dimension. They often contained a single nucleus, as in the upper cell of Fig. 20, but frequently they contained two or more, up to six in a single cell. In all these cells the neutral red material was present as exceedingly fine, dust-like particles. In most of the cells the particles were so minute that they could not be made out individually. Between them the cytoplasm had a faint, brownish red cast, as if the dye had either diffused into the cytoplasm itself or had stained some substance there situated. On the other hand, the hazy, diffuse staining may have been due to an optical effect brought about by the great number of the small bodies staining with the neutral red. These cells usually contained many fat droplets.

Cells such as these are illustrated in the accompanying drawings. Fig. 20 shows two cells which were found in a shred of subcutaneous tissue taken from an animal which had received two injections of nitrogen over a period of four days. The larger cell contained two nuclei and the rosette of neutral red vacuoles was readily seen. The smaller cell, partly hidden by the larger, had less neutral red but showed the same grouping of minute neutral red bodies. The smaller cell also contained fat droplets.

Fig. 10 shows a large binucleate cell from an animal which had had daily injections of oxygen for seven days. The neutral red vacuoles in this cell were all minute, quite regular and equal in size, and were clear and distinct. No diffuse reddish brown tint was present about the vacuoles as was commonly seen in the majority of these cells. The neutral red bodies formed two distinct rosettes between the nuclei. Several fat droplets were visible in the peripheral portions of the cytoplasm.

The cells shown in Figs. 12 and 13 were obtained from animals which had received nitrogen over a period of ten days and four days respectively. The first of these cells had three nuclei with definite nucleoli. The neutral red vacuoles were dust-like in character, stained diffusely brownish red, and simulated a rosette in their arrangement. The second cell, with two nuclei, was a younger form. Its cell border appeared irregular but the neutral red pattern was quite similar to that of the other cell.

Fig. 11 illustrates the most usual appearance of these cells. This particular cell was very large, approximately twenty-five microns

differences. The neutral red bodies, for example, were more numerous, smaller in size, and more uniform in shape; fat droplets were often visible in the cytoplasm; and the cells were generally larger than the typical monocyte. Such cells, therefore, were not normal. They resembled those shown in Figs. 1 and 2, Plate 1, of the paper by Cunningham, Sabin, Sugiyama, and Kindwall,<sup>12</sup> and we may call them, with these authors, "modified monocytes." They were the characteristic cells of the exudates, being present in great numbers following injections of oxygen and nitrogen, but appearing less frequently after the administration of carbon dioxide.

A few of the "modified monocytes" are shown in Figs. 1, 5, 6, 7, and 9. These particular cells, drawn directly from supravital preparations, were found in the scrapings of the subcutaneous tissues of an animal which had received daily oxygen injections over a period of four days, but they represent the predominating type found following the injection of all three gases. The tendency on the part of the neutral red bodies to form a rosette in many of the cells is well shown. Occasional fat droplets may also be seen. Such droplets were usually at the periphery of the cell, but they were sometimes found above or below the nucleus, or even among the neutral red vacuoles. They were most numerous in the cells from animals which had received carbon dioxide and least numerous following oxygen injections. They appeared commonly after six or eight days, but even then only the older cells were involved.

Cells of the same general character as those already described but containing several nuclei were also present in varying numbers. They were rare at periods earlier than four days, but they appeared about that time and from then on they increased in number and often in complexity of structure. At eight or ten days they were invariably found. Figs. 3 and 8 show such cells, one with two nuclei, the other with three. These cells, like the mononuclear forms described above, showed the characteristic grouping of neutral red vacuoles about a centrosphere. Fat droplets, too, were often numerous.

In addition to the abundant cells of the types already described there appeared another type of cell. Cells of this type were few in number in the early stages (three to five days) but they gradually increased until the tenth or twelfth day when they were present in considerable quantities, especially in the nitrogen and oxygen



Only rarely were cells found in the process of direct division and never in indirect. Fig. 14, shows a giant cell which was dividing directly to form two daughter cells, one with three nuclei, the other with one. In Fig. 21 there is a cell from an oxygen animal of four days in which direct division was taking place. In both of these dividing cells there was a fairly equitable division of the neutral red bodies between the daughter cells, especially in the second one where distinct rosettes were present in association with each nucleus.

An interesting phenomenon observed repeatedly in the preparations from oxygen animals, and occasionally with nitrogen, was the arrangement of the cells about gas bubbles which were often present in the fluid, Figs. 16 and 17. While these structures were frequently found, it is to be presumed that they were more numerous in the actual tissues than they were in the stained preparations, for undoubtedly during manipulation of the exudate many of these structures were destroyed. About the gas bubbles, which were of varying sizes, there were groups of typical monocytes, sometimes few, more often many. In the first illustration three cells are seen about such a bubble of gas. These cells had extended, sucker-like processes which were closely applied to the edge of the vacuole. The cells themselves had quite definite rosettes of neutral red and a few small neutral red bodies were present in the processes, in some cases extending down to their tips.

The second drawing, Fig. 17, shows a somewhat larger gas vacuole which was surrounded by many cells arranged in spoke-like fashion about the bubble. The cells nearest the gas space had applied to its edge a large portion of their surfaces while those more remote had extended long cytoplasmic processes to the edge of the vacuole. All these cells contained small amounts of neutral red often scattered in the cytoplasm, though generally forming a small but distinct clump.

In preparations from animals which had received oxygen and nitrogen injections for four or more days, cells of the granulocytic series were only occasionally seen. Polymorphonuclear neutrophiles were the cells most usually found, but their presence was so infrequent as to call for special mention. With carbon dioxide, on the other hand, polymorphonuclear neutrophiles were more commonly seen, and occasionally these cells predominated. Such cells when present in any of the preparations were quite typical in character,

in diameter, and contained five nuclei arranged in a circle about a dense mass of fine, hazy granules of neutral red. It was present in the scrapings from the subcutaneous tissue of an animal which had received four injections of nitrogen over a period of fourteen days. Such cells were not uncommon following nitrogen and oxygen injections, but were rarely found in carbon dioxide animals. They usually contained varying numbers of fat droplets which were almost always present in the peripheral parts of the cytoplasm. Phagocytized foreign material was rarely seen.

Fig. 15 shows a cell from the subcutaneous tissue of a carbon dioxide animal which had received daily injections of the gas for twelve days. While there were many minute bodies of the dye massed in the central portion of the cytoplasm of this cell, there were also many larger vacuoles, some of which stained intensely with the neutral red, thus indicating an acid reaction. This type of cell usually contained many fat droplets.

Fig. 14, from an eight-day nitrogen animal, shows one of these giant cells in the process of direct division. The neutral red material was present in very small vacuoles and these were found not only in the individual cells but in the long strand of cytoplasm which connected them.

All of these large cells, both mononuclear and multinuclear, resembled the typical epithelioid cell of the tubercle, as this cell appears in supravitality stained preparations. The increased size of the cell, the dust-like character of the neutral red vacuoles, their arrangement in a rosette-like mass in the cytoplasm and the presence of fat droplets in varying numbers are all characteristic of the epithelioid cell of true tubercle. In these preparations such epithelioid cells rarely contained large vacuoles of neutral red or other recognizable phagocytized material.

Between the relatively normal monocytes, which were present in small numbers, and these typical epithelioid cells, there was the great group of modified monocytes which made up the largest percentage of the cells. These appeared to be intermediate in character, being larger than the typical monocytes and containing more neutral red vacuoles. Indeed all stages of the transition could be followed, from early modified forms up to late stages where the neutral red bodies were minute, granular and hazy, and the cells quite large.

The evidence of active multiplication of these cells was not great.

Most of these exudative cells contained small groups of mitochondria arranged peripherally to the neutral red vacuoles, though sometimes these structures could be seen scattered between the neutral red-stained bodies.

Fixed connective tissue cells were not infrequently seen to be dividing indirectly, so that active proliferation of these cells was in progress. In no case, however, were monocytes found in the process of cell division.

In addition to monocytes and epithelioid cells, clasmotocytes were found more frequently in the tissues than they were in the exudates. Such cells resembled those seen in the smears. They were more numerous in specimens from carbon dioxide animals than they were in preparations from animals which had received oxygen or nitrogen.

It was not unusual to find in preparations of tissue a tendency on the part of certain connective tissue cells to become rounded up and hypertrophied. Such cells were at first broad and tapering, with one or more long cytoplasmic processes which often connected them with adjacent cells. They contained varying numbers of small neutral red vacuoles which were usually scattered in the cytoplasm, but which sometimes simulated a rosette. In some of these cells the neutral red was present in small amount, only a few vacuoles somewhat larger in size than those of the modified monocyte being present. But in others the intracytoplasmic structures showed a definite resemblance to those of the free monocyte, even to the grouping of fine mitochondria outside the rosette. Fig. 18 shows such a group of connective tissue cells, and illustrates the usual distribution of neutral red vacuoles in their cytoplasm. Two typical, slightly modified monocytes were present between these cells. This group of cells came from an animal which had had daily oxygen injections for a period of seven days.

In another seven-day oxygen animal an analogous, though more advanced, change was noted (see Fig. 19). Here a group of six or eight cells, apparently connective tissue cells, appeared to be in the process of rounding up and becoming free. Most of these cells had long, strand-like, cytoplasmic processes which extended from the cell into the adjacent tissue. The cells themselves, except for the single processes, were almost oval in shape and contained many small vacuoles of neutral red. Two of the cells had become com-

were usually motile and not uncommonly contained one or more neutral red vacuoles of varying size and of a deep reddish tint.

In addition to the cells described above, certain large phagocytic cells were noted sporadically in all of the preparations, but were more common in the carbon dioxide animals than in the others. Such cells were large, irregular in shape, with an oval nucleus. Neutral red vacuoles of varying size and tint were scattered irregularly throughout the cytoplasm. There was no tendency to rosette formation or fine granulation. Phagocytized material, such as nuclear fragments or even whole leukocytes, was not limited to the peripheral cytoplasm, but was usually stored deep within the cell. These large phagocytic cells, however, differed greatly from the monocytes and epithelioid cells and were so infrequently found that their total percentage in several differential counts was never more than 1 to 3 per cent. They corresponded to the "clasmatocytes" as described by Sabin, Doan and Cunningham<sup>16</sup> and pictured in their Plate I.

In addition to the living cells described above there were often dead or disintegrated cells which either stained diffusely or failed to take up any of the dye. These were more numerous in the carbon dioxide animals than in the others. No attempt was made to identify such cells.

*Tissue Spreads:* Spreads of thin sheets of subcutaneous connective tissue showed typical elongated, sometimes stellate, connective tissue cells of the fixed tissue. In general, these cells did not take up any of the neutral red, but where double stains were used they exhibited small numbers of minute, granular mitochondria grouped about the nucleus. Many strands of collagen were also visible, running in all directions. Present within such fragments of tissue, often clumped in clear spaces between collagen fibers were varying numbers of the typical monocytes and modified monocytes of the exudates as well as occasional larger cells which approached the epithelioid cell type. Epithelioid cells as such were rarely found, although the two cells in Fig. 20 came from a tissue spread of a four-day nitrogen animal. In general, the younger forms of modified monocytes predominated, especially after oxygen and nitrogen injections. In the carbon dioxide animals polymorphonuclear neutrophils were also in evidence and the monocytic types were distinctly fewer than they were in animals injected with the other two gases.

in the others. Following oxygen injections they were frequently found, but with carbon dioxide they were few in number and usually small in size. Present within most of these spaces there was a scattered granular deposit which consisted undoubtedly of albuminous material which had been precipitated from the edematous fluid noted grossly in the tissues. The presence of such a precipitate within almost all of these vacuoles which were originally made by gas indicates that the gas was not, at the time of fixation at least, the only occupant of the spaces, but that fluid which exuded from the surrounding tissues was also present in considerable quantity. In many places there were circular, clear areas within the precipitate, the evidence of gas vacuoles which had been present in the fluid itself. For the sake of convenience in referring to these gas-formed spaces in the tissues, regardless of whether or not they contain fluid, they will hereafter be spoken of as "gas spaces."

In addition to the albuminous precipitate there were in the gas spaces varying numbers of cells and occasional irregular strands of an intensely red-staining substance which in some places resembled old fibrin, but which in others appeared to consist of stretched and fragmented strands of collagen fibers which had become separated from the tissue and were undergoing certain degenerative changes by which they became transformed into hyaline-like masses. The accumulations of cells and hyaline material were generally found scattered here and there within the spaces. They were most common at the edges of the spaces, Figs. 22 and 24, where the fluid had been abundant. Occasionally they were present in spaces which showed no evidence of fluid.

The typical cells of the exudates found in the gas spaces are shown in Fig. 23. They were so numerous that one cannot but feel that they were identical with the monocytes and modified monocytes observed in the supravitality stained preparations. Such cells resembled the so-called large mononuclear leukocyte. They were irregularly round or oval, with single, eccentrically-placed, curved or indented nuclei. In fixed preparations nothing more specific could be made out. These cells were present in varying numbers within the gas spaces. They appeared also in the surrounding tissues. They were the characteristic cells of the exudates, appearing almost to the exclusion of other types. Polymorphonuclear neutrophilic leukocytes were rarely seen, except in the carbon dioxide animals where

pletely separated from the tissue and were gradually becoming changed into rounded, free cells. One of these had a typical rosette. At the periphery of this small group there was a considerable number of free monocytes or modified monocytes.

From a morphological standpoint, these altered connective tissue cells seemed to be undergoing definite transition from the fixed, tissue type to the free type. During the period of examination of the supravital preparations, those connective tissue cells which were apparently normal at first contained no neutral red, but they gradually acquired red-stained vacuoles which, at the end of an hour, were often quite numerous. These probably represent a degenerative change taking place within the cell during the time of observation. On the other hand, the cells which were undergoing the transitional change contained their vacuoles from a very early period, thus indicating that in some way they were already different from the normal connective tissue cells. Such altered cells appeared as types transitional in character, intermediate between the fixed connective tissue cell and the free cell of the exudate, the monocyte. It must be borne in mind, however, that the evidence of this apparent transition rests at present solely on a morphological basis.

Such apparent transformations from fixed to free cells were observed chiefly following oxygen injection. With nitrogen and carbon dioxide there were fewer evidences of a similar change. Granulocytes were not commonly found in tissue spreads except in carbon dioxide animals, where they were seen in large numbers.

It was not uncommon to find small blood vessels, especially capillaries, in the tissues studied. In no case was there any evidence of phagocytic activity on the part of the endothelial cells which formed their linings, nor was there any neutral red in their cytoplasm. No endothelial cells were seen in the process of cell division. There was no apparent desquamation. In such capillaries the endothelial cells formed a smooth, flat layer which lined the vessels and showed no evidence of becoming free or wandering into the tissues.

### FIXED TISSUE STUDIES

Sections of tissues fixed in Zenker's fluid and stained with hematoxylin and eosin showed varying numbers of large and small spaces which were more numerous in the nitrogen animals than they were

found following administration of all of the gases, but were especially numerous in animals which had received nitrogen. Figs. 30 and 31 show two of these cells, both from nitrogen animals. The epithelioid character of these cells was not so easily demonstrated, for exactly similar cells were not observed in the living state. Some of them, however, from their similarity to the free giant cells, and from their resemblance to the giant cells of a tubercle, may reasonably be assumed to be of the epithelioid type.

In Fig. 31 a single cell is seen. This was definitely of the Langhans type with a rim of nuclei extending part way about the elongated cell. One border of the cell was in contact with the gas space. Fig. 30 shows a similar cell in which the nuclei were grouped in the part of the cell farthest removed from the accumulation of gas. A single cytoplasmic process projected out toward the space which contained nitrogen.

One of the largest giant cells observed is seen in Fig. 39, from a ten-day oxygen animal. This cell, which bordered on a small gas vacuole contained over forty visible nuclei. These formed a triangular zone about a pale, somewhat granular central area. Adjacent to this cell was another of similar character but with fewer nuclei. Occasionally large giant cells were found about masses of degenerating collagen. Whether or not such cells were of monocytic or clasmatocytic origin was not determined.

The tissues about the spaces in the oxygen and nitrogen animals showed definite changes. These were not always present following carbon dioxide administration but were observed with sufficient frequency to indicate that at certain periods there was a more pronounced reaction than at others and that the duration of the tissue change was shorter with carbon dioxide than with the other gases. Where any of the gases had been recently injected the connective tissue showed evidence of marked proliferative activity and appeared to be loose and areolar in character. Scattered throughout such foci there were considerable numbers of large mononuclear cells. Often varying amounts of a bright red, homogeneous, hyaline substance were also seen. This substance was accumulated most commonly at the edges of the gas spaces themselves, but some of it was situated within the newly formed tissue. This material resembled fibrin, and indeed much of it reacted as such when special staining methods were employed.

they were uniformly present. These large mononuclear cells varied in size. The smallest were approximately 8 to 10 microns in diameter, while the largest were from 15 to 30 microns. In twenty-four to forty-eight hour animals they were found only occasionally, but after four days their presence was constant.

Associated with these cells, which always predominated, there were sometimes others which were long and spindle-shaped. The latter cells resembled, morphologically, connective tissue cells from which, indeed, they may have originated. On the other hand, they may have been separated or desquamated cells from the walls of the gas spaces. In either case they appeared to be living cells at the time of fixation. Probably some of them corresponded to those connective tissue cells which, when studied in the living state were seen to be undergoing an apparent transition from fixed cells into free cells of the exudate.

Giant cells with two, three, or even more nuclei were not uncommon in the exudate within the gas spaces. Such cells were usually round or oval and in many of them the nuclei were arranged in ring-like fashion about the center of the cell. These giant cells resembled those which were often found in supravital smears which had been fixed and stained after the studies on the living cells had been completed. All of the giant cells found free in the gas spaces were undoubtedly the same as the large multinucleate epithelioid cells which were observed in the supravital preparations. Nothing in their character or appearance, however, would lead one to suspect them of being epithelioid in type. But when the giant cells of the fixed supravital smears were carefully studied it was found that in many of them the neutral red vacuoles could still be made out, somewhat faintly to be sure, but clearly enough to enable one to determine that such cells had been epithelioid giant cells in the supravital preparation. There can be little question, therefore, that the free giant cells of the exudate, as observed in tissues which had not been stained with neutral red, were in fact multinucleated epithelioid cells. In Fig. 40, there is a clump of large giant cells from a gas space in the tissues of an animal which had been given nitrogen for a period of two months.

In addition to the giant cells which were free in the exudate there were in the sections others which were a part of the tissue itself. These were adjacent to, or bordered on, gas spaces. Such cells were



that they projected into the lumen of the vessel. Occasionally a mitotic figure was visible. There was no evidence, however, that any of these cells ever left the vessel wall, became phagocytic, or wandered into the adjacent tissues.

With carbon dioxide, as we have previously stated, all of the tissue changes were less striking than they were with the other gases. In general the connective tissue was not loose and web-like in character, but appeared quite dense. Gas vacuoles were not numerous, unless the animal was sacrificed within a very few hours after the last injection. Edema was less marked, exudative cells were fewer, and the whole reaction to the gas was less pronounced. Polymorphonuclear neutrophilic leukocytes were usually seen in considerable numbers, and their presence differentiated the reaction to this gas from that to either oxygen or nitrogen. Occasionally, however, vacuoles were numerous and large mononuclear cells were present in great numbers.

The connective tissue in the oxygen and nitrogen animals was not always of the thin, areolar, proliferating character already described. In many places the tissues were compact and dense. Such foci were more common in animals which had received injections of gas for a week or longer, and it is thought that they represent true scars in which the fibrous tissue which had proliferated about gas spaces had become contracted and hyaline. Cell proliferation was no longer active. In such areas gas spaces often persisted, but they appeared to be quite old. They were separated from each other by thick strands of dense connective tissue similar to that just described (Fig. 28) and free cells were less numerous, either within the spaces or in the tissues about them. As time went on the dense, fibrous walls grew thicker, forming ultimately stiff, hyaline membranes which separated gas accumulations, as in Fig. 29 from a sixty-day nitrogen animal.

Gas spaces were to be observed almost everywhere in the subcutaneous tissues of the oxygen and nitrogen animals. These were often very small and vacuole-like, but variations from such minute spaces up to bubbles which measured 2 to 4 mm. in size were noted. When the vacuoles were recent in their formation they contained much albuminous material and the tissues about them were thin, edematous and areolar in nature, and the borders of the spaces often consisted of a brightly red-staining, hyaline membrane which re-

While the cells which were present in these tissues varied somewhat in size they all resembled those of the exudate in the gas spaces. Sometimes giant cells with two or more nuclei were present, but they were less common here than they were in or near the gas vacuoles. The connective tissue cells themselves were large and distinct, usually appearing to be spindle-shaped but not infrequently being branched or stellate in form. Their nuclei were round or oval, but occasionally were irregularly indented. With the hematoxylin-eosin stain their cytoplasm acquired a faint bluish tint and in general showed no structure other than a delicate reticulation. Such cells were frequently seen in the process of indirect division and mitotic figures were relatively numerous. About the hyaline masses described above, the proliferation of connective tissue cells was especially active.

Often it seemed as if some of the fixed cells were actually forming free cells, for there were many places in the sections where apparent transitions from the one cell to the other were taking place. Such changes were most striking at the borders of the gas spaces where certain of the cells accumulated in the wall of the vacuole and appeared to be separating themselves from the tissue, much as the living cells in Fig. 19 were doing. Adjacent to, or intermingled with these cells which maintained but loose contact with the tissue and yet in many respects still resembled the connective tissue cells, there were free, rounded cells which were the same as those of the exudate. The inference that changes such as these represent a transition from connective tissue cell to free, large mononuclear cell rests on morphological evidence alone. But augmented by the findings in the supravital preparations, the inference seems to be justified in part, at least. Certainly the connective tissue cells themselves were undergoing rapid proliferation, and many of them exhibited a morphological change from the normal cell type into that of the large mononuclear. The complete proof that there was a true transformation must await further investigation. The morphological observations here recorded merely suggest the possibility of such a transition.

The endothelial cells of the blood vessels in these areolar tissues were sometimes actively multiplying. This was especially true in the oxygen animals, less so in those which had received nitrogen and carbon dioxide. Such endothelial cells often became rounded up so

tion of elastic fibers, torn, wavy strands of old elastic tissue, usually coarse and thick, were found intermingled with the collagen.

Although in places the homogeneous material which lined the gas spaces or the hyaline fragments which were present within them was actually derived from preëxisting connective tissue, in other places such accumulations were composed of fibrin. Sometimes the fibrin formed the lining of the spaces especially when they were recent, Fig. 26. This lining often resembled the hyaline membrane found by Wolbach in the air spaces in emphysematous tissue. Sometimes it was present as scattered fragments within the gas spaces. And not infrequently it was situated in the tissues just outside the older gas spaces, surrounded by newly formed connective tissue which was apparently replacing it, Fig. 27. In this latter situation it formed the membrane-like layer which resembled the elastic lamina of arteries.

Where fibrin was present in the tissues it was occasionally possible to demonstrate long, thin fibrils of elastin which showed no evidence of rupture. These were in the immediate neighborhood of the fibrinous accumulations. They were so delicate, and their relationship to proliferating connective tissue cells so intimate, that it was difficult not to conceive of them as newly formed. But we have no other evidence of the actual production of elastin.

Sometimes, as has been noted, fibrin or altered collagen formed the actual lining of gas spaces. This was true when the vacuoles were recent or new. At later periods, however, cells grew out and covered the hyaline membrane, thus transforming many of the gas spaces into cysts lined with endothelial-like cells and often containing some fluid. The new lining cells were sometimes large and rounded up, especially where the tissues were folded or bent, but more often they were flat, Figs. 28 and 29. They appeared first along the border of the hyaline material, as can be seen in places in Fig. 26. Gradually they grew out to cover it up, ultimately forming a layer of cells which was sometimes quite thick, Fig. 27.

Spaces lined with flat cells were first seen three to four days after the beginning of the injections, and as time went on they became more numerous. In Fig. 28, from an oxygen animal of eight days, and in Fig. 29, from an animal which had had nitrogen injections for over two months, portions of such spaces are seen. Morphologically, the lining cells formed a membrane which resembled an

sembled the hyaline fragments found within some of the spaces and described above. When the vacuoles were older they were surrounded by dense fibrous strands which were lined with flat cells, as in Figs. 28 and 29. Such vacuoles usually contained but little fluid, but sometimes there was evidence that much had been present. Occasionally in the tissue just outside the lining of some of the spaces there were irregular masses of a red-staining substance which resembled that found in abundance in the tissues about the recently formed vacuoles. This material often partly or completely surrounded many of these older gas spaces, forming a more or less continuous membrane which resembled the fenestrated elastic lamina of the arterial wall, Fig. 27.

The nature of the deep reddish homogeneous material which was found in so many situations could not be determined from a study of hematoxylin-eosin preparations alone. Special staining methods were therefore employed. With Mallory's anilin blue stain some of this material stained intensely red and in no way resembled collagen which stains uniformly a bright blue color. In other places, however, there was evidence of collagen in some of the red-staining masses, but the collagen fibers appeared to be old rather than newly formed. They seemed to be undergoing a degenerative change in which the fibrils became fused and hyaline. They often had no connection with the surrounding tissue but lay free in the gas spaces, as in Fig. 22. With phosphotungstic acid hematoxylin there was again a distinction between the various accumulations of this material. Some of it took a deeply purplish tint and stood out sharply from the paler, amber-colored surrounding tissue. In other places, however, the homogeneous strands tinctorially resembled collagen, acquiring a yellowish brown color and appearing in some places quite homogeneous while in others it was faintly fibrillar.

Some of the red-staining material noted in the hematoxylin-eosin preparations, therefore, was probably derived from collagenous strands which were stretched and distorted by the gases and often separated from the tissue. With anilin blue this collagen seemed to be degenerating but with phosphotungstic acid hematoxylin there was little except its homogeneous nature to differentiate it from collagen elsewhere. That collagen was the source of some of this fibrin-like material was further evidenced by the fact that when these tissues were stained by Verhoeff's method for the demonstra-

cumulation of cells was undoubtedly about a vacuole of gas and in many ways it resembled the arrangement of cells about gas vacuoles in the supravital preparations. In another animal which had been injected with oxygen for ten days several small tubercles were seen in the tissue. One of these is shown in Fig. 35.

In a third animal which had received daily injections of oxygen for twelve days, a larger nodule was found, Fig. 36. The typical cells within this lesion resembled those which were seen in the gas spaces described above. Almost without exception they were large mononuclear cells which were grouped about a small, clear central area adjacent to which was a giant cell. Most of the cells were not phagocytic but within a few of them there were small, darkly staining granules which are visible in the illustration. There was no evidence of gas in the structure, although the minute clear zone in the center may represent either the edge of a gas space which was above or below the level of the plane of section, or the remains of a vacuole from which the gas had been removed by the cells.

Fig. 38 illustrates the structure which most simulated a tuberculous lesion. It consisted of an elongated space, presumably caused by the gas (nitrogen). About the space there was an accumulation of large mononuclear and giant cells. The similarity to a tubercle was so striking that the isolated lesion might have been easily mistaken for one caused by the tubercle bacillus. The giant cells were all of the Langhans type and, with the mononuclear cells, they surrounded the gas space in the same manner that monocytes surround the caseous material of a tubercle.

## DISCUSSION

By the subcutaneous injection of three gases, oxygen, nitrogen, and carbon dioxide, we have been able to bring about the local production of monocytes, modified monocytes, and epithelioid cells. Monocytes and modified monocytes were found in great numbers at the end of two to four days following the beginning of oxygen and nitrogen administration, and they were constantly present after that time. Carbon dioxide also stimulated the production of these cells, but to a lesser degree than did the other two gases. After three or four days epithelioid cells began to appear and were found in increasing numbers as the period of gas injection was prolonged.

endothelial or serous lining. It was often impossible, however, to differentiate the cells from the typical connective tissue cells which they simulated closely. Tintorially they differed slightly, for their cytoplasm was stained more deeply blue than was that of the connective tissue cells, thus exhibiting a slightly more basophilic reaction.

The lining cells are seen under higher magnification in Figs. 32 and 34. Their endothelial or serosal character is there evident, and their active multiplication as lining cells is indicated by the presence of mitotic figures. Gas spaces lined by such cells were found with great regularity, and in no case was there evidence that the cells came from any tissue other than the adjacent connective tissue which usually formed a dense wall which grew thicker as time went on. The possibility of their origin from endothelium lining minute blood vessels or lymphatic spaces in the tissues could not, however, be definitely ruled out, even though prolonged search revealed no evidence of the outgrowth of endothelial cells. The fact that some of the spaces contained fluid may indicate that they were connected in some way with the local lymphatics and that the lining cells came from lymphatic endothelium. In the supravital preparations there was found a peculiar grouping of monocytes about gas vacuoles, especially oxygen, occasionally nitrogen. In the fixed tissues such a grouping of cells about gas bubbles was also noted. Fig. 25 shows a mass of large mononuclear cells about a clear zone which presumably contained gas. The structure was found in the tissues of an animal which had received oxygen injections over a period of six days. It was present in one of the larger gas spaces which contained considerable fluid as was indicated by the granular precipitate about it. The radiating character of the cells was less evident here than in the living preparations, but the grouping was thought to be significant. In Fig. 37 a similar structure is seen. This resembles more closely those observed in living preparations.

Structures resembling true tubercles have also been found. In one animal which had received daily injections of carbon dioxide for three days there were many large mononuclear cells which lined some of the gas spaces and which arranged themselves at right angles to the wall, much as epithelioid cells do about a tubercle. In another place in the same section there was a tubercle-like structure in which the radiating character of the cells was most striking. This ac-

The latter cell they consider to be present in connective tissue everywhere, although it is most common in the spleen, bone marrow and lymph nodes. In their study of the rôle of the monocyte in tuberculosis, Cunningham, Sabin, Sugiyama, and Kindwall express the belief that the effect of the tubercle bacillus is to cause a local increase in the number of reticular cells and then definitely to force them toward the production of monocytes. In scrapings of connective tissue septa from a normal lung they were able to find reticular cells in very small numbers, but in specimens from the lungs of tuberculous animals the reticular cells were present in much greater numbers and they tended to form monocytes exclusively. The increase in the number of reticular cells in tuberculous infections and the tendency of these cells in their maturation to form monocytes alone, they attributed to some chemical influence affecting the cells through the surrounding medium, for neither reticular cells nor young monocytes were found to contain the organisms. This concept they confirmed to some extent by studying the effect of injections of various fractions of the tubercle bacillus.

Mallory,<sup>18</sup> on the other hand, makes no distinction between different types of large mononuclear cells. He classifies them all as "endothelial leukocytes," and in his opinion they are derived from endothelium, either locally or at some distant point. It is these "endothelial leukocytes" that form the characteristic cells of the tubercle, both epithelioid cells and giant cells.

By others, notably Maximow,<sup>19</sup> the monocytes are thought to have their origin in a multipotential cell which is present in the connective tissues. Still others believe that they originate from a fixed connective tissue cell. Indeed Carrel and Ebeling,<sup>20</sup> studying the monocyte in tissue culture observed the reverse of the last process, for they assert that monocytes, under artificial conditions can be transformed into fibroblasts. There is thus a wide variety of opinions as to the origin of the monocyte, but as yet there is not enough evidence to permit of any final decision.

In the normal connective tissue we may consider the following cells or cell types to be present. First, the connective tissue cell which is found everywhere: this is the cell commonly called the "fibroblast," usually long and spindle-shaped, but not infrequently branching or stellate in form. By it collagenous and elastic fibers, and fibroglia are considered by some to be formed. Second, the

Finally giant cells, many of them similar to the Langhans cell of the tubercle, were observed, appearing first at the end of about a week and becoming more numerous, larger in size and more complex in structure as time went on. Were it not for the absence of tubercle bacilli, it would have been difficult in many cases to determine that the cells had not come from a tuberculous lesion.

The reaction reached its height at the end of eight to ten days, for at that time the maximum number of cells of all types was found. At later periods giant cells were larger and contained more nuclei, but the other cells remained about the same in number and in character. With oxygen and nitrogen these cells were found almost to the exclusion of cells of other types. Polymorphonuclear neutrophilic leukocytes and clasmatoocytes were only rarely observed. With carbon dioxide, however, the total number of cells was less, and although monocytes predominated, there were also more neutrophilic leukocytes and clasmatoocytes than were observed with the other gases.

The presence of monocytes, modified monocytes, epithelioid cells and giant cells was determined by studying both tissue scrapings and tissue spreads by means of the supravital method for staining living cells. In sections of the fixed tissues stained with hematoxylin and eosin the predominating cells were large mononuclear cells, morphologically indistinguishable from the large mononuclear leukocytes of the blood. Giant cells were also found. One may reasonably assume that such cells in the fixed tissues were the same as the monocytes, modified monocytes and epithelioid cells which were found in the living preparations.

The production of great numbers of monocytes, modified monocytes, and epithelioid cells by the subcutaneous injections of such simple irritants as the three gases mentioned above, brings up the question of the origin of these cells. Do they arise locally from some cell or cells in the connective tissue which under appropriate conditions can give rise to the monocyte? Or do they come to the sites of gaseous infiltration from the blood stream having been formed elsewhere in the body? We have reason to believe that the monocytes, in greater part at least, arise locally.

Cunningham, Sabin, and Doan<sup>17</sup> believe that the monocytes, as well as all of the other white cells of the blood, come from a common primitive blood cell which itself is derived from the reticular cell.



modified monocytes were found. In preparations of fixed material it was also not unusual to find many free cells, apparently from the tissues, bordering on gas spaces and even on the verge of becoming completely separated. The local transformation of connective tissue cells into monocytes would explain the presence of the latter cells in such great quantities at the sites of injection.

In Figs. 18 and 19 there are shown cells which we interpret as connective tissue cells which are undergoing transition into monocytes. In the first of the illustrations the cells are seen in the early stage of the transformation. In the second illustration, showing a late stage of the change, the usual morphology of the tissue cells is entirely lacking. The cells are well rounded up, save for thin threads of cytoplasm which still connect some of them with the parent tissue. Others are entirely free. In some of these cells there is a tendency to rosette formation on the part of the neutral red bodies, and in one cell which has lost all contact with the tissue the rosette is well formed. Morphologically these cells are now almost identical with the cells of the exudate.

From stained histological sections there was also evidence of the change from fixed tissue cells to free mononuclears, though one could make fewer inferences here than were possible in the supravital preparations. Almost everywhere about the recently formed gas spaces, the connective tissue cells were proliferating in great profusion. The stimuli for such widespread proliferation may be, first, the destruction or death of connective tissue cells which have been injured mechanically; and second, the presence of fibrin which has not been removed by the cells of the exudate. That destruction of connective tissue cells took place is evident in the sections, and much of the proliferative activity on the part of uninjured cells can be ascribed to the process of regeneration. Fibrin was also present here and there in the sections, but it was never found in large quantities, and certainly could not account for the tremendous activity of the connective tissue cells which were far removed from it. On the other hand, cell proliferation was constantly very active in the tissues at the edges of the gas spaces. Here it was not infrequently noted that large mononuclear cells accumulated, and it is not unreasonable to assume that such cells actually originated in those situations. Were it not for the further evidence brought out in the supravital preparations, however, it would be impossible to make

reticular cell, believed by Sabin and her co-workers to be a primitive cell which gives rise to the white cells of the blood. Mallory and Parker,<sup>21</sup> on the other hand, do not admit of its separation from the "fibroblast," and they speak of "reticular cell" and "fibroblast" as identical. Third, the wandering phagocytic cell, (the "macrophage" of Evans,<sup>22</sup> the "histiocyte" of Aschoff and Kiyono, the "clasmatocyte" of Sabin *et al.*, the "endothelial leukocyte" of Mallory), classified by Mallory as belonging to the large mononuclear cell group, but separated from the monocyte by Sabin, Doan, and Cunningham on the basis of its reaction to supravital dyes. And finally, the endothelial cell which forms the lining of blood and lymph spaces. By Mallory and his school this cell is considered to be the parent cell of the free phagocytic cells.

In our preparations of the living cells and tissues supravitaly stained, we were able to see apparent transitions from a fixed connective tissue cell to the monocyte. The tissue cell appeared to be identical with the common connective tissue cell or "fibroblast." The earliest evidences of a change in this cell were: first, the appearance of small neutral red bodies within the cytoplasm near the nucleus, and second, the tendency on the part of the cell so altered to become contracted and rounded up. The vacuoles appeared as distinct structures very soon after the preparations were made. They were yellowish brown in color and resembled the vacuoles of the monocyte. Usually they were grouped near the nucleus, but were not infrequently scattered throughout the cytoplasm. When the preparations had stood for some time the vacuoles increased in number. At a later stage the tendency of the cell to round up became more pronounced and the neutral red bodies often assumed a rosette-like arrangement. In the final stage of the transformation the cells gradually became separate entities quite free from the tissue, each with a single oval or slightly curved nucleus and containing a mass of relatively small neutral red vacuoles, usually arranged in a rosette and located in the hof of the nucleus. Such cells resembled the typical monocyte.

In general such transitional types were found at the edge of a mass of connective tissue, and the impression gained was that they were in the process of becoming separated from the tissue. This condition might have easily existed at the edges of gas spaces, for it was within these spaces that such great numbers of monocytes and

preparations at least, the former cells were the most important source of the latter. The changes which took place during the process of transition have been described. It has been indicated that the epithelioid cells were large, contained dust-like neutral red bodies, and were often multinucleate.

The cause for the transition from monocyte to epithelioid cell or epithelioid giant cell is not easy to determine. That the transition occurs we feel certain. Such a change might be the result of a stimulus which acted either from the outside or from the inside of the cell, such as the chemical alteration of the medium about the cell or the presence within its cytoplasm of some substance or substances taken up by it. In this connection it is interesting to note that carbon dioxide, which disappears most rapidly from the tissues, is the gas which is least active in producing the characteristic changes described. Nitrogen, on the other hand, which remains longest in the tissues is the most potent of the three gases in the production of epithelioid cells. Oxygen, both in its rate of removal and in its power to produce epithelioid cells lies between carbon dioxide and nitrogen. It must be borne in mind, however, that nitrogen is highly soluble in lipoidal substances and that its effect in producing epithelioid cells may bear some relation to this property. We have no evidence either for or against such a possibility. On the basis of our observations, however, we feel justified in saying that since all three gases are capable of producing epithelioid cells, since their potency in the production of these cells varies approximately in inverse ratio to the rate at which they are removed from the tissue, since they are all normally present in living cells, and since they are in no way related physically or chemically to each other, our evidence favors the view that the activity of these gases in causing the transformation of monocytes into epithelioid cells is not dependent upon their gaining access to the interior of the cells.

The fact that oxygen, nitrogen, and carbon dioxide are all effective even if in different degree, in the production of monocytes and epithelioid cells, indicates that these cells are not the result of a special type of irritant. The gases are elemental, or almost so, and although the number of cells and the amount of change in the cells differ quantitatively from the reaction in tuberculosis, the difference is one only of degree and not one of character. If the work of

such an assertion. We feel that the weight of evidence in our material, both living and fixed, indicates the local formation of the monocytes and suggests strongly the possibility of their formation, in part at least, from some type of fixed connective tissue cell.

The reticular cell as defined by Sabin, Cunningham, and their co-workers was not recognized in the supravital preparations. Apparently, unlike the tubercle bacillus the gases introduced did not produce a great initial increase in these cells. This lends further support to the view that the cells arising locally sprang from some other type of parent cell. In our opinion, as already stated, this other cell is the connective tissue cell itself. In fixed histological sections the reticular cell could not be identified.

In the experiments reported here there was no evidence that monocytes, as such, originated from the wandering phagocytic cell, or "clasmatocyte," of the connective tissues. The latter cell was readily recognized, although it appeared but rarely, and in none of the preparations was there any evidence of transitions from one type to the other.

The endothelial cell likewise seemed to play no part in the formation of the monocytes. Occasionally, especially in animals which had received oxygen, these cells showed evidence of proliferative activity, and they were often rounded up so that they projected into the lumen of the vessel. But no evidence was found that they became separated from the vessel wall or wandered into the tissues. Apparently their multiplication was brought about by injury to the vessel wall, and their activity was wholly regenerative in nature. Rarely, in regions where there was active connective tissue proliferation about masses of fibrin they were also found in the process of division. Even in these situations there was no evidence that they became transformed into free cells.

In our experiments the only irritants used were simple gases. Their action was to cause not only the production of great quantities of monocytes, but also in some way to cause them to undergo certain changes which resulted in the formation of epithelioid cells and epithelioid giant cells. The nature of these changes is not clear, nor are we as yet able to say that epithelioid cells can not be derived from some other cell type as, for example, the clasmatocyte. But we do feel that the large number of transition forms between monocytes and epithelioid cells which have been found indicates that, in our

from them by a thin layer of newly formed tissue. This was the lamella-like structure which was thought to resemble the fenestrated elastic coat of an artery.

By means of Verhoeff's stain for elastic tissue it was shown that wherever old collagen was present it was not unusual to find considerable numbers of torn or frayed elastic fibers which stood out sharply from the surrounding tissue. Unquestionably these strands resulted from the rupture of preëxisting elastic fibers, for they were coarse and thick. Where the tissue cells were proliferating about masses of fibrin, however, long, thin, delicate strands of elastin were often seen in the new tissue. These differed markedly from the coarse, wrinkled, wavy fragments found in the older tissue and described above. They appeared to be newly formed, a definite part of the new tissue in which they were present. It is possible that under the conditions of unusual tension due to the continued presence of gas, the connective tissue cells may be stimulated to produce elastin. Definite proof of the new formation of elastic tissue cannot be given here, but the findings strongly suggest such a possibility.

When the gas spaces had existed for a considerable period of time, as for example, six days or longer, their walls became dense and fibrous, and were lined with flat, mesothelial-like cells which formed a continuous membrane about the individual spaces. The origin of these cells presented a difficult problem. Connective tissue, as far as we know, does not usually form the lining of a normal space, except, according to Mallory,<sup>23</sup> on the inner surface of the dura where, in his opinion, the so-called "dural endothelium" consists of a layer of flat connective tissue cells. Clarke,<sup>24</sup> however, believes that a flat, mesothelial-like lining may be formed by connective tissue cells about accidental spaces caused by introducing solid foreign bodies (celloidin, paraffin) into the tissues. He holds also that flat cells lining serous surfaces or blood vessels "may regenerate from deep connective tissue cells and do not necessarily arise from adjacent intact mesothelial or endothelial cells."

In the subcutaneous tissues of our animals the only fixed tissue cells available to form a lining to the gas spaces were endothelial cells from the neighboring blood and lymph vessels, or the connective tissue cells themselves. In sections of the fixed tissues it was possible only to identify the lining layer as such, for little could be determined about the type of the individual cells. The only

Campbell, McIver, Redfield and Benedict, and others, be considered then we must feel that the non-specific character of the reaction is quite definite, for these investigators have shown that when any of the above-named gases are injected into the subcutaneous tissues or into a natural cavity they remain but a short time before they contain, mixed with them, other gases which have come by diffusion from the blood. Thus the reaction observed in our experiments is not one which came as a response to a single pure gas, but to a gaseous mixture which varied in each animal.

Wolbach, in his brief description of the air spaces in emphysematous mediastinal tissues, mentions a thin, fibrinous membrane which lined the spaces. This membrane resembled that which he found lining many of the alveoli and alveolar ducts in the lungs of patients who had died of pneumonia following influenza. There is no description of a cellular exudate in the emphysematous spaces or in the tissues about them. In fixed preparations from our animals we frequently found in sections stained with hematoxylin and eosin various deposits of an intensely red-staining, hyaline, fibrin-like substance. This material was found in various situations. Often it appeared as fragments in the gas spaces. Occasionally it lined the spaces, forming a thin, flat membrane similar to that described by Wolbach. Frequently it was incorporated in the tissues about the gas spaces. The nature of this substance could not be determined in preparations stained by the routine method, and various special stains were employed. It was then found that the hyaline material stained sometimes like fibrin, sometimes like collagen. A double origin was therefore indicated, some coming from the tissue fluids under conditions which could readily result in the formation of fibrin, and some coming from collagen fibers which had degenerated and formed fused, hyaline strands. The isolated fragments which were present in the gas spaces stained as often like fibrin as they did like collagen. The thin, lining membranes also gave the two reactions, though in the majority of cases they gave the reaction for fibrin. Not infrequently it was possible to see flat or spindle-shaped cells growing out along the borders of such fibrinous membranes. These cells gradually surrounded the fibrinous layer so that in later stages the fibrin was isolated as a sort of hyaline membrane surrounding, or partly surrounding, the gas spaces but removed

and as the time of exposure to the gases became longer these cells underwent changes similar to those which are known to occur in true tubercle. The reaction to the gases was found to differ only in degree from that which characterizes tuberculosis.

Not infrequently structures which resembled the anatomical tubercle in form were observed. In the supravital preparations, for example, accumulations of modified monocytes were seen about small vacuoles of gas. Two such masses are shown in Figs. 16 and 17. These structures exhibited a radiating collection of cells which extended pseudopodial processes toward the edge of the vacuole, thus simulating to some degree the form of the tubercle. The gas vacuole occupied a place comparable to that occupied by necrotic or caseous material in the tubercle. These structures were apparently free in the fluid which was present in most of the gas spaces. They were not a part of the tissue.

In histological sections from some of the animals accumulations of cells similar to those noted above were sometimes seen, as in Figs. 25 and 37, but they were not numerous. On the other hand, deep within the tissues themselves there were many lesions which approached the tubercle in their histological structure. Such lesions are seen in Figs. 35, 36, and 38. The first is quite small and indefinite, but suggestive. The second contained no gas but consisted of an accumulation of large mononuclear cells and one giant cell which resembled those found in the gas spaces. In our opinion this cellular structure probably formed about a gas vacuole. The gas had either been removed before the time of death of the animal, or the accumulation of cells represented a tangential section through the edge of a vacuole. Fig. 38 shows a structure which resembles still more closely the lesion of tuberculosis. Epithelioid-like cells could be recognized; and giant cells, almost all of the Langhans type, were present. The relationship of this lesion to a gas space was evident. Lesions such as these found in the affected tissues indicate that the gases injected, in addition to producing cells which supravitally stained resemble those of tuberculosis, are also capable of bringing about tissue changes which histologically resemble the anatomical tubercle.

recognizable difference between the lining cells and the connective tissue cells that lay beneath them was that the cytoplasm of the former was more basophilic than that of the connective tissue cells. It was not possible to demonstrate the formation of collagen or elastin about such lining cells as were studied in specially stained preparations.

Many attempts were made to discover outgrowths of endothelial cells to form the space lining, but nothing to suggest an endothelial origin was found in the sections. Occasionally red blood cells were seen in the gas spaces which were lined with the flat, cellular membrane, but since these could have come in as a result of hemorrhage at the time of autopsy, their presence did not help to prove the endothelial character of the lining. Certainly there was no evidence that blood was circulating within the spaces. Fluid, however, was sometimes present, as indicated by a granular precipitate. This might favor the view that such cavities had become lined with endothelium from lymph channels and were therefore a part of the lymphatic system. We have no other evidence, however, in favor of this concept. That these lining cells came from the various cells of the exudate does not seem probable because of the difference in their reactions to neutral red. For the moment the nature of the cells which lined these older gas spaces must be left undetermined. The evidence that they may have originated from connective tissue cells, especially in the light of Clarke's observations, can not be cast aside.

Since these studies were carried out in conjunction with work now going on in various centers on the tissue and cellular reactions in tuberculosis, it is important to relate the findings in our experiments to those found in studying the disease itself. By the simple subcutaneous injection of various gases we have produced in large numbers the cells characteristic of this disease. At the very earliest periods, twenty-four to forty-eight hours, polymorphonuclear neutrophilic leukocytes were found in considerable numbers. These may be considered as coming out in response to the primary mechanical injury, for after forty-eight hours they were practically absent, except in animals which had received carbon dioxide. Their presence in such numbers following injections of this gas must bear some relation to the gas itself or to its effect on the tissue cells. With the other gases, however, the cellular response was almost entirely a monocytic one,



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## SUMMARY AND CONCLUSIONS

1. By the subcutaneous injections of oxygen, nitrogen, and carbon dioxide there have been produced large numbers of monocytes, modified monocytes, epithelioid cells, and epithelioid giant cells which resemble those of tuberculosis as seen in supravital preparations.
2. The causes for this reaction are non-specific.
3. Monocytes are considered to arise locally, originating from some type of fixed connective tissue cell.
4. Epithelioid cells and epithelioid giant cells appear in these experiments to arise almost entirely from monocytes, the cause of the transformation being evidently due to some chemical change in the medium about the cell.
5. Histological structures resembling true tubercles have been found in considerable numbers.
6. Fibrin is present in the gas spaces, often forming a thin membranous lining.
7. The new formation of elastic tissue is suggested about gas spaces where tissue cells are under tension.
8. Spaces lined with flat, mesothelial-like cells and containing both gas and fluid are found after six or eight days. The lining cells are thought to originate from connective tissue cells, although endothelium can not be ruled out as a source.

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FIG. 12. Multinucleate epithelioid cell from a ten-day nitrogen animal.

FIG. 13. Binucleate epithelioid cell from a four-day nitrogen animal.

FIG. 14. Large, multinucleate epithelioid cell apparently in the process of direct division. Note fine neutral red granules and their distribution not only in the individual cells but also in the long strand of cytoplasm which connects them.

FIG. 15. Large cell from twelve-day carbon dioxide animal. This cell contains, in addition to many fine, brownish red granules which resemble those of the monocyte, still larger masses which stained with varying shades of red. Some of the latter were pale pink in color, others bright red. Such cells were quite common following carbon dioxide injections but were not seen in preparations from oxygen and nitrogen animals. This cell is apparently of monocytic origin.

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## DESCRIPTION OF PLATES

Figs. 1 to 21 are drawings made from living cells stained with neutral red. The red vacuoles are shown in black and the occasional fat globules are shown as circles. The cells shown in Figs. 1 to 9 were found in the exudates of animals which had received gas injections for a period of four or more days and are characteristic of the cells found in such animals. They were fewer in number following carbon dioxide than after the administration of oxygen or nitrogen.

Figs. 22 to 40 are photomicrographs made from sections of tissue removed at autopsy and prepared in the usual manner.

### PLATE 18

FIG. 1. Modified monocyte from subcutaneous tissues of guinea pig which had received daily injections of oxygen for four days. Note small granules of neutral red grouped in rosette formation. A few fat droplets are present.

FIG. 2. Modified monocyte of large size from a four-day oxygen animal. The rosette is formed by very small granules of neutral red.

FIG. 3. Binucleate modified monocyte from a four-day oxygen animal. The neutral red bodies are larger than in the preceding cells.

FIG. 4. Large modified monocyte which borders on epithelioid type. From an oxygen animal of four days. Neutral red vacuoles are very small and are more numerous than in the other cells.

FIGS. 5-9, incl. Group of five modified monocytes from a four-day oxygen animal. One cell has three nuclei and a small mass of foreign material.

FIG. 10. Large, binucleate, early epithelioid cell from a seven-day oxygen animal. The neutral red vacuoles are small, granular, and quite regular in size. Two distinct rosettes are present. A number of fat vacuoles are scattered in the peripheral cytoplasm.

FIG. 11. Large epithelioid giant cell from a fourteen-day nitrogen animal. This cell is characteristic of the majority of epithelioid giant cells found after the injection of oxygen and nitrogen. It was not commonly seen following carbon dioxide administration. Note the great mass of dust-like neutral red granules in the center of the cell. Fat vacuoles are present in small numbers.

## PLATE 19

FIG. 16. Small bubble of gas (oxygen) surrounded by three modified monocytes which extended sucker-like processes of cytoplasm to be applied to the edge of the vacuole. The cells contain neutral red rosettes and small granules of neutral red are seen in the cytoplasmic processes. A few fat droplets are also seen in each cell.

FIG. 17. A larger bubble of oxygen from the same preparation as the preceding, showing many modified monocytes arranged in radiating fashion about the vacuole. Both of these structures occurred in animals which had received oxygen for seven days. In its form this structure suggests the arrangement of epithelioid cells about the periphery of a tubercle.

FIG. 18. A group of connective tissue cells which show the early stage of the transformation of these cells into monocytes. The cells have more cytoplasm than normal and considerable numbers of small neutral red vacuoles are present. There is also a tendency on the part of the affected cells to become wider than usual. Many of them are still connected with adjacent cells by thin strands of cytoplasm. Two monocytes are present between the cells.

FIG. 19. A later stage in the transformation of fixed tissue cells into monocytes. Here the cells are well rounded up and are connected with the tissue by single strands of cytoplasm. The neutral red vacuoles appear smaller than in the previous preparation and a tendency to rosette formation is noted. Several of the cells have become completely detached and in one of them there is a distinct rosette. Two typical modified monocytes are also present.

FIG. 20. Two large epithelioid cells from a four-day nitrogen animal. These were found in a tissue spread. Fine granulation of the neutral red material is noted and rosette formation is distinct. The lower cell has two nuclei.

FIG. 21. Monocyte dividing by direct division. Daughter cells contain distinct rosettes. Fat droplets are present in the cytoplasm. Complete separation of the cells has not occurred.

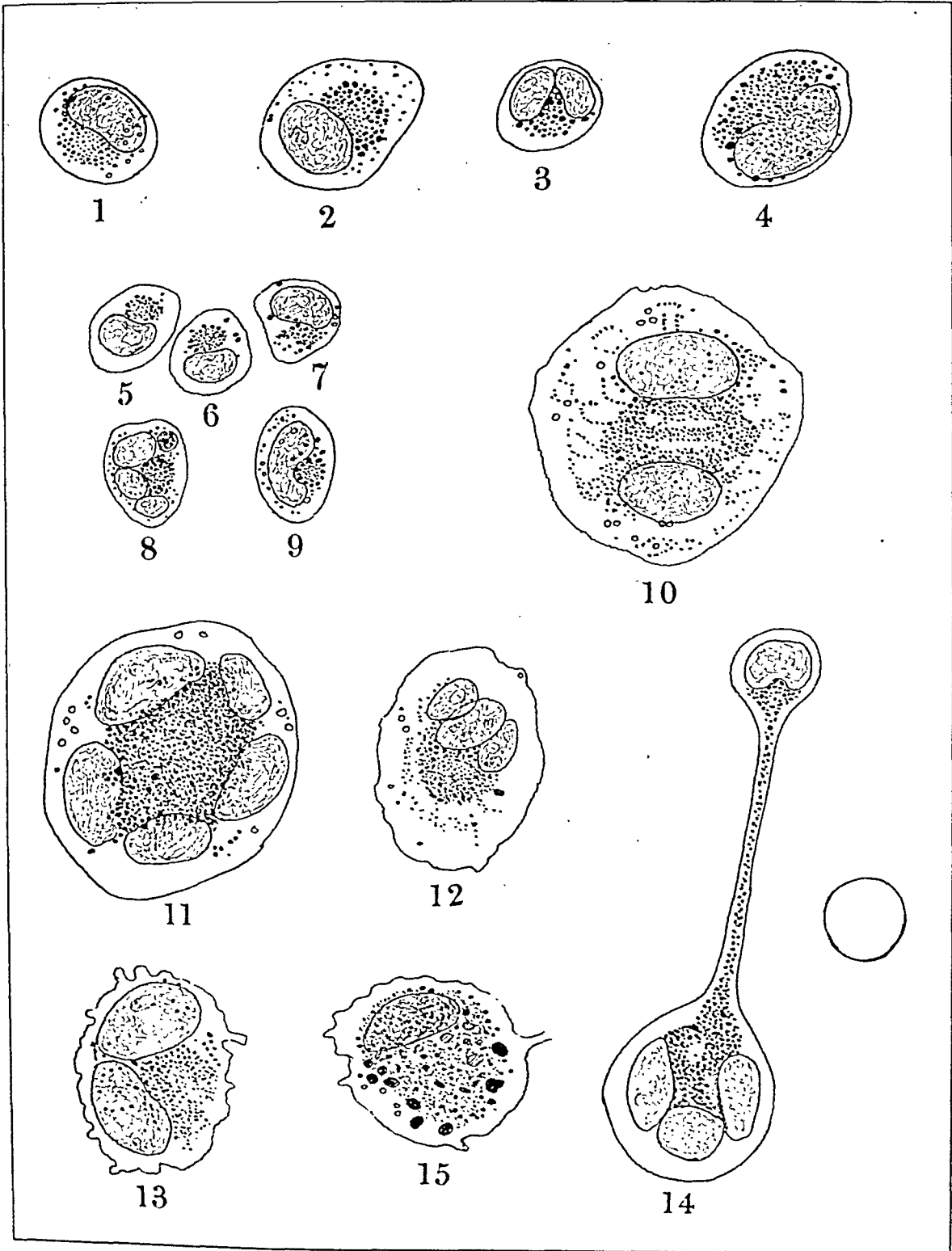


PLATE 20

FIG. 22. Photomicrograph of a gas space in an animal which had received oxygen injections for twelve days. Note the many cells, the albuminous granules, and the fragments of tissue which in places is becoming hyalinized. Phosphotungstic acid hematoxylin stain.  $\times 150$ .

FIG. 23. Photomicrograph of cells from same animal as above. All the cells are large mononuclears, resembling the large mononuclear leukocytes of the blood. These cells are undoubtedly the same as the monocytes and modified monocytes found in supravital preparations. Phosphotungstic acid hematoxylin stain.  $\times 1350$ .

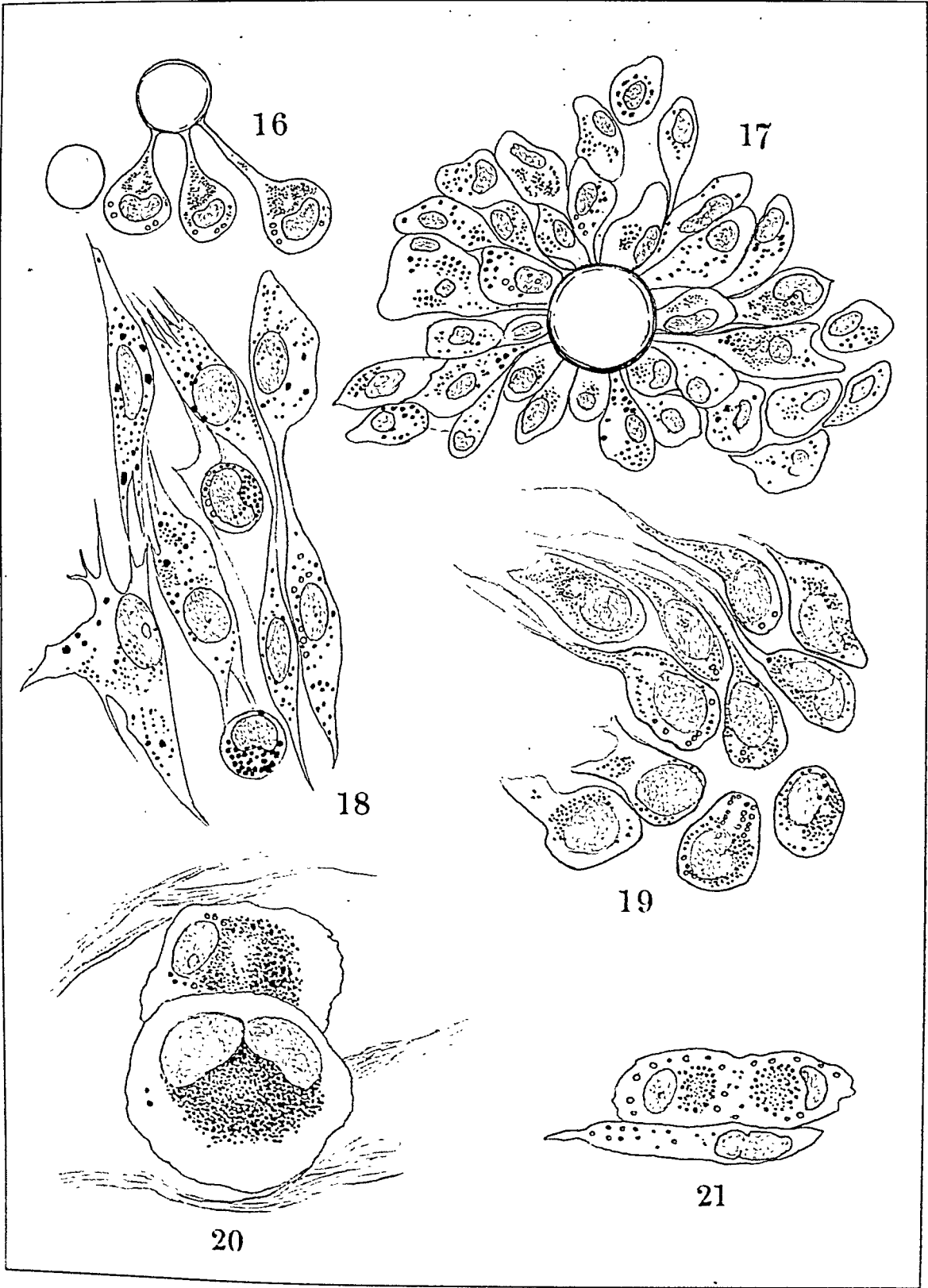




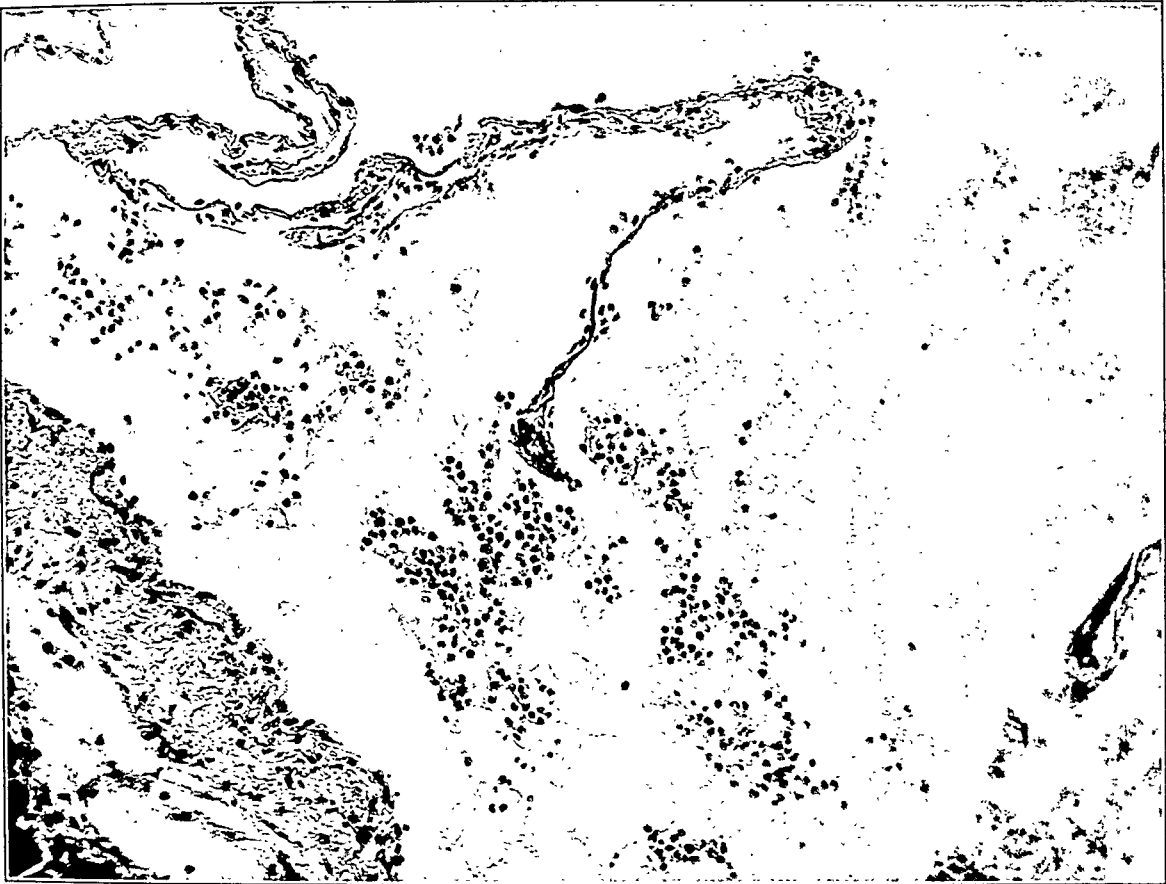
PLATE 21

FIG. 24. Photomicrograph from animal which had received oxygen for twelve days; to show typical appearance of newly formed gas spaces. Note groups of cells within the spaces and the evidence of fluid. The edges of the spaces are lined with thin strands of fibrin. The surrounding connective tissues are thin and edematous and there is active proliferation of tissue cells. Hematoxylin and eosin stain.  $\times 60$ .

FIG. 25. Photomicrograph of a group of large mononuclear cells about a clear space (gas). This group was found in a large gas space of an animal which had received oxygen for six days. There are many albuminous granules about the structure, indicating abundant fluid. The structure resembles those found in the supravital preparations, Figs. 16 and 17. See also Fig. 37, a higher magnification of a similar, but smaller, structure. Hematoxylin and eosin stain.  $\times 480$ .

FIG. 26. Photomicrograph of the edge of a recently formed gas space to show the thin lining membrane of fibrin which was not infrequently found in these situations. The fibrin is closely adherent to the tissues and in the lower part of the illustration it blends imperceptibly into strands of old collagen. Note the edematous condition of the spaces and the tissues surrounding them. Phosphotungstic acid hematoxylin stain.  $\times 310$ .

FIG. 27. Photomicrograph of thin, homogeneous layers of fibrin becoming incorporated into the tissues about a gas space. Here the fibrin is quite old and hyaline. About it connective tissue cells have proliferated separating it completely from the space. Such wavy strands of fibrin resemble the elastic coat of an artery. Phosphotungstic acid hematoxylin stain.  $\times 150$ .



22



23

PLATE 22

FIG. 28. Photomicrograph of strands of connective tissue separating old and compressed gas spaces. Note how the tissue is becoming thick and dense. Cell proliferation has practically ceased. Note also the layer of flat or somewhat spindle-shaped cells which form the lining membrane of the gas spaces. In places these cells or others just beneath them are in the process of indirect division. These cells are thought to originate from the underlying connective tissue cells. Hematoxylin and eosin stain.  $\times 310$ .

FIG. 29. Photomicrograph of a portion of a strand of connective tissue separating old gas spaces in an animal which had received nitrogen injections for a period of two months. Note the greatly increased thickness of the fibrous wall, the dense, hyaline character of the connective tissue and the absence of exudative cells. The flat cells which line the gas spaces resemble those in the preceding figure although they are less regular. Mitotic figures were not infrequently found in these cells. Hematoxylin and eosin stain.  $\times 310$ .



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Wright

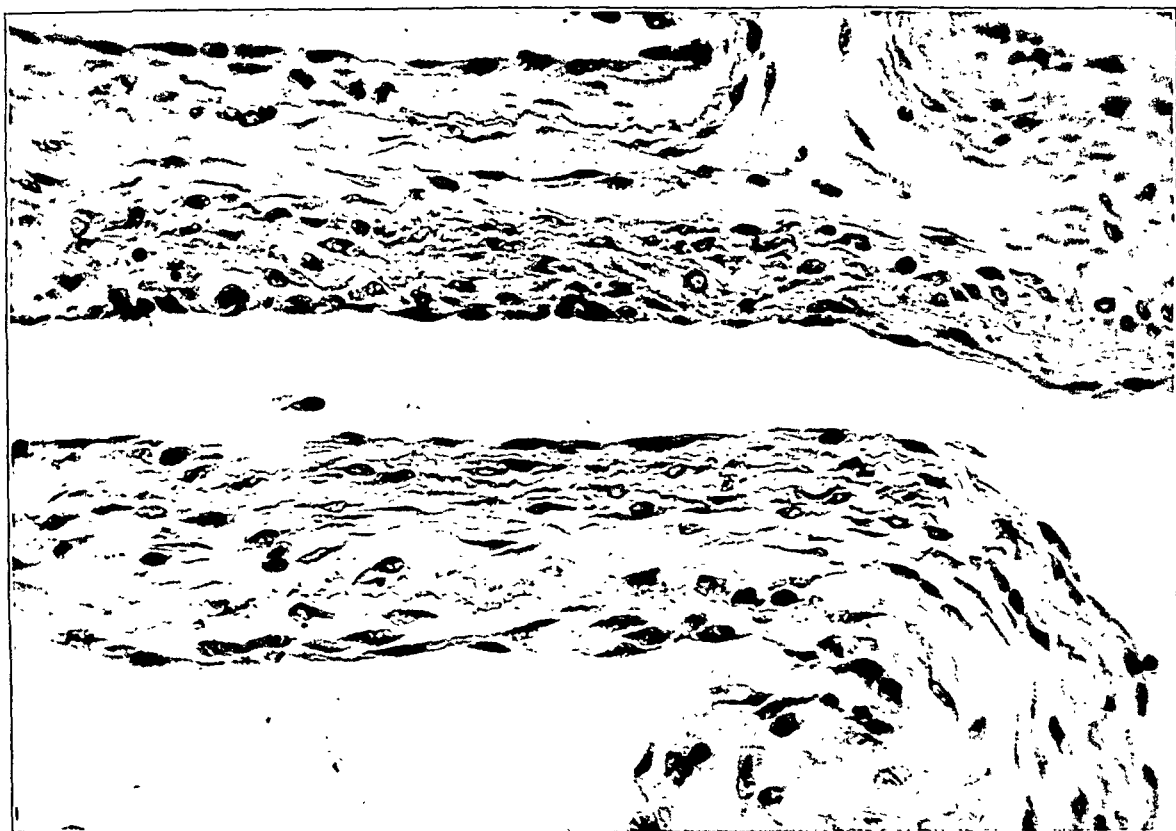


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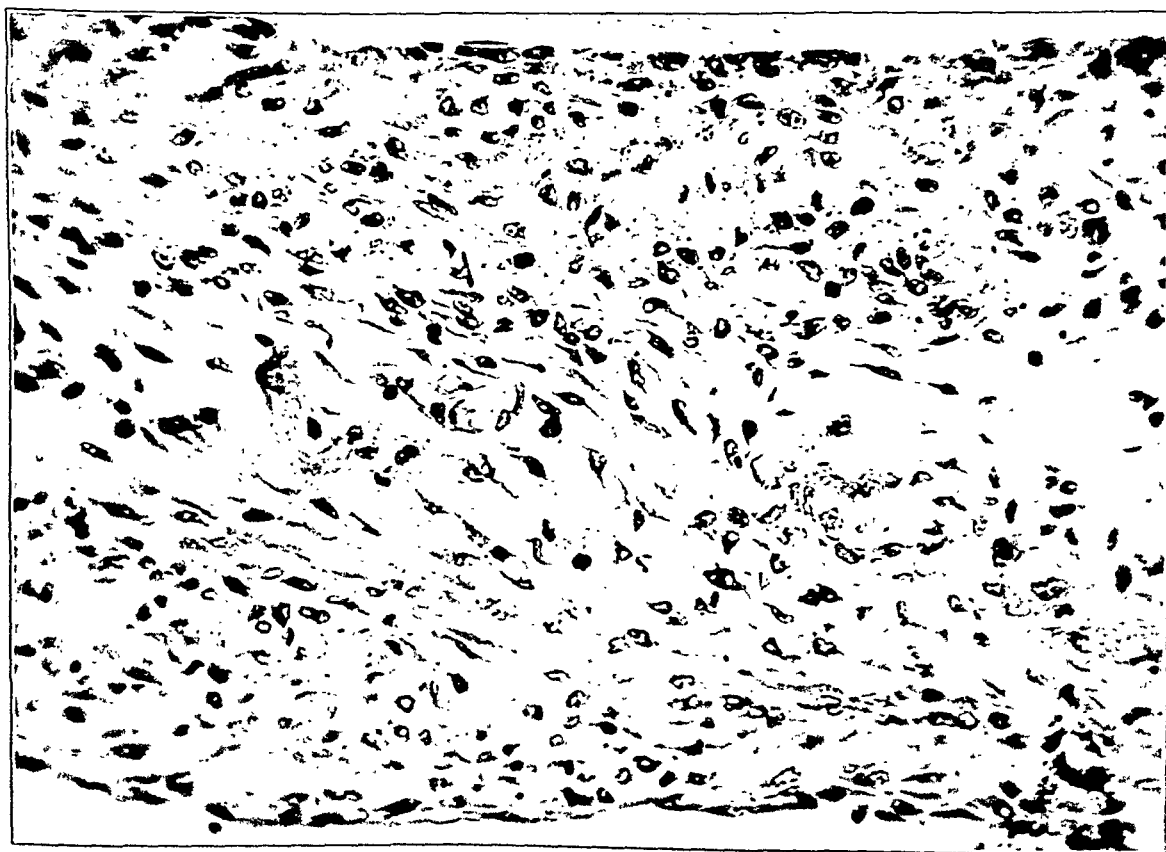
Injection of Gases into Subcutaneous Tissues

PLATE 23

- FIG. 30. Photomicrograph of a large giant cell of Langhans type at edge of a gas space. From an animal which had received nitrogen injections for eight days. The nuclei are arranged in the part of the cell which is farthest removed from the gas space and a cytoplasmic process projects toward the gas vacuole. Hematoxylin and eosin stain.  $\times 480$ .
- FIG. 31. Photomicrograph of a giant cell at the border of a gas space, from an eight-day nitrogen animal. This cell, though flattened, resembled the Langhans type of cell. Hematoxylin and eosin stain.  $\times 480$ .
- FIG. 32. Photomicrograph of a mitotic figure in one of the flat or spindle-shaped cells which form the lining of an older gas space. Hematoxylin and eosin stain.  $\times 1100$ .
- FIG. 33. Photomicrograph of a large cell at edge of gas space, showing triple mitosis. This suggests that some of the multinucleate or giant cells might have been formed as a result of such abnormal types of cell division. Hematoxylin and eosin stain.  $\times 1100$ .
- FIG. 34. Photomicrograph of flat lining of older gas space, showing one cell in process of indirect division. Hematoxylin and eosin stain.  $\times 1100$ .



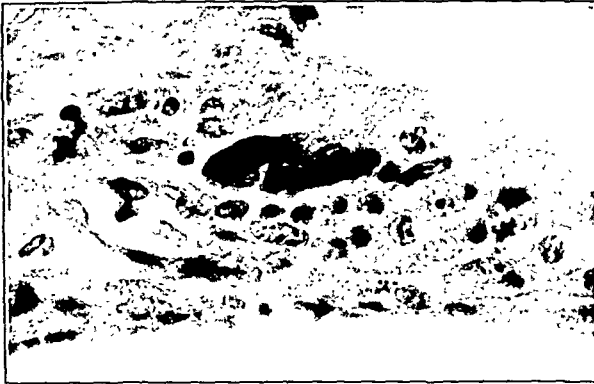
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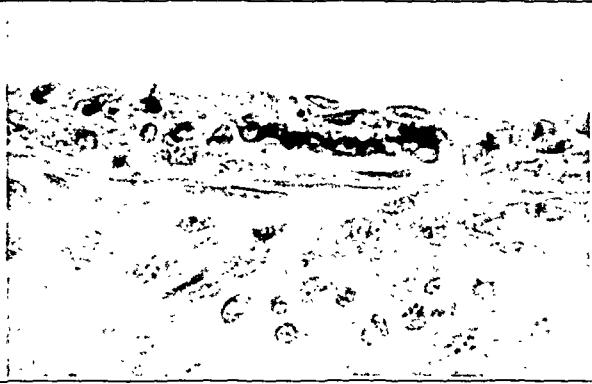
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PLATE 24

- FIG. 35. Photomicrograph of a tubercle-like structure consisting of an accumulation of large mononuclear cells in connective tissue between muscle fibers. The lesion appears to have formed about a gas vacuole, the remains of which may still be seen. Two or three giant cells are present. From an animal which had received oxygen injections for ten days. Hematoxylin and eosin stain.  $\times 480$ .
- FIG. 36. Photomicrograph of a small, round, tubercle-like nodule consisting of an accumulation of large mononuclear cells and a single giant cell. Some of the cells contain phagocytized material. The clear zone in the center suggests that the lesion developed about a gas space. The distribution of the cells suggests an "onion-layered" arrangement. Hematoxylin and eosin stain.  $\times 480$ .
- FIG. 37. Photomicrograph of an accumulation of large mononuclear cells about a gas vacuole. From a twelve-day oxygen animal. This, like Fig. 25, resembles the arrangement about gas vacuoles noted in the supravital preparations. Some of the cells in this illustration appear to radiate from the gas space. Phosphotungstic acid hematoxylin stain.  $\times 1350$ .
- FIG. 38. Photomicrograph of a lesion which closely resembles a tuberculous process. Here there is an accumulation of large mononuclear and giant cells about a gas space. From an eight-day nitrogen animal. Note the numerous giant cells and the resemblance of many of them to the typical giant cells of true tubercle. Phosphotungstic acid hematoxylin stain.  $\times 150$ .



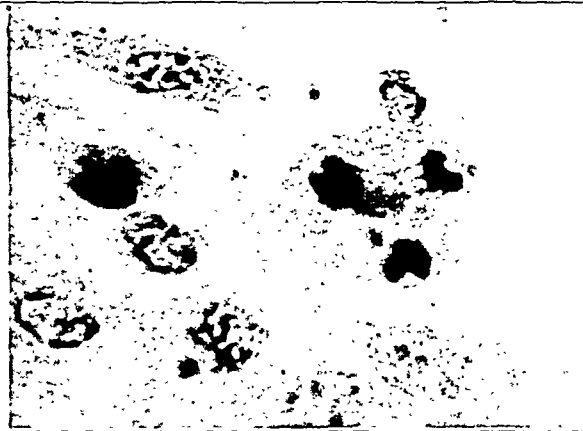
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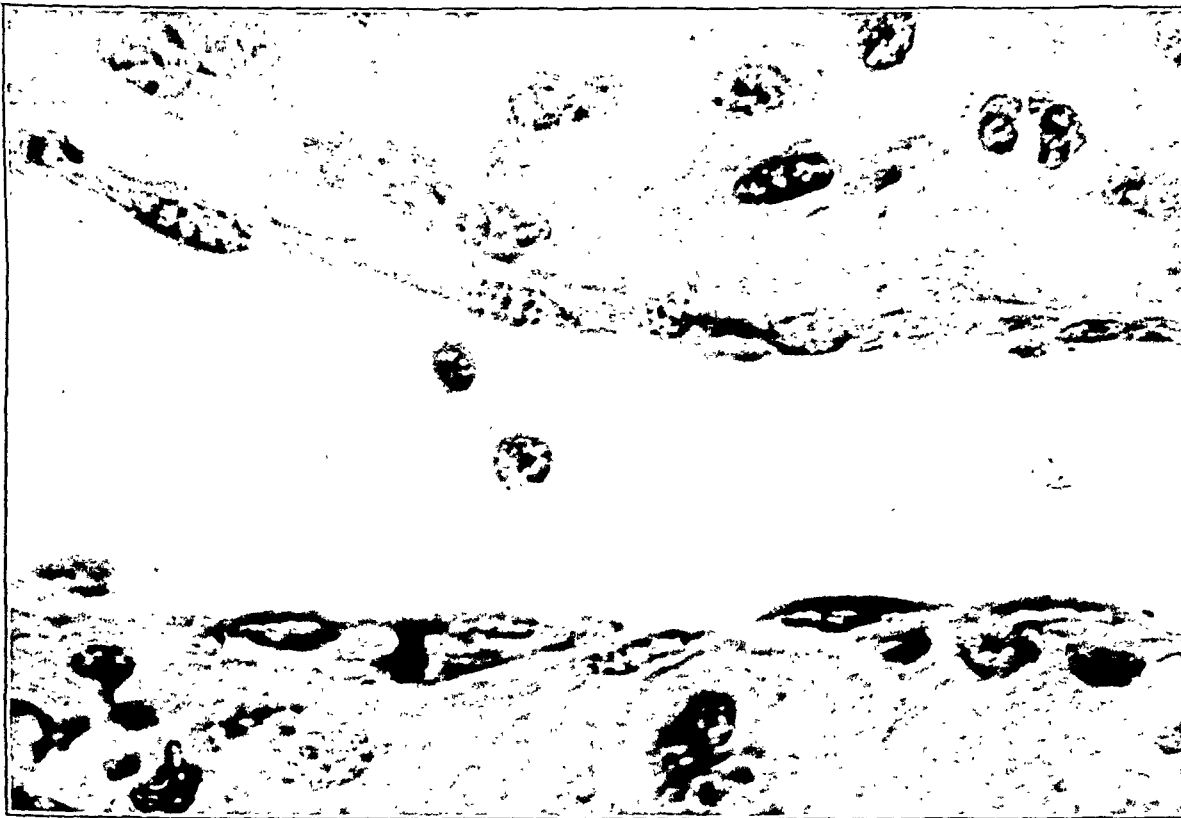
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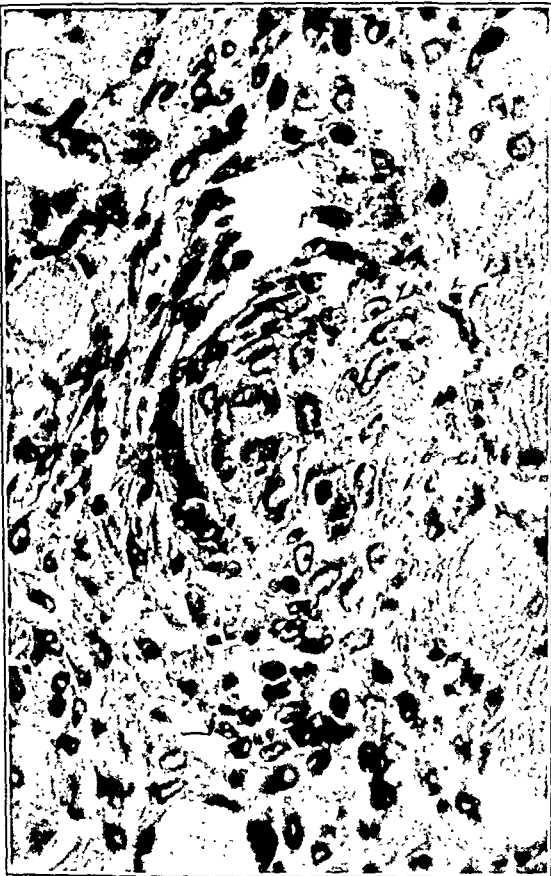
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PLATE 25

FIG. 39. Photomicrograph of a large giant cell in the tissues at the edge of a gas space from a ten-day oxygen animal. More than forty nuclei were counted in this cell. It is one of the largest observed in any of the preparations. Note the arrangement of the nuclei about a clear space. This resembles the central region in many of the epithelioid giant cells observed in the supravital preparations. In the latter cells the central zone usually contained countless numbers of dust-like neutral red bodies. Hematoxylin and eosin stain.  $\times 1100$ .

FIG. 40. Photomicrograph of a group of large giant cells found in a gas space from an animal which received nitrogen for a period of two months. Each cell contains many nuclei. Hematoxylin and eosin stain.  $\times 630$ .



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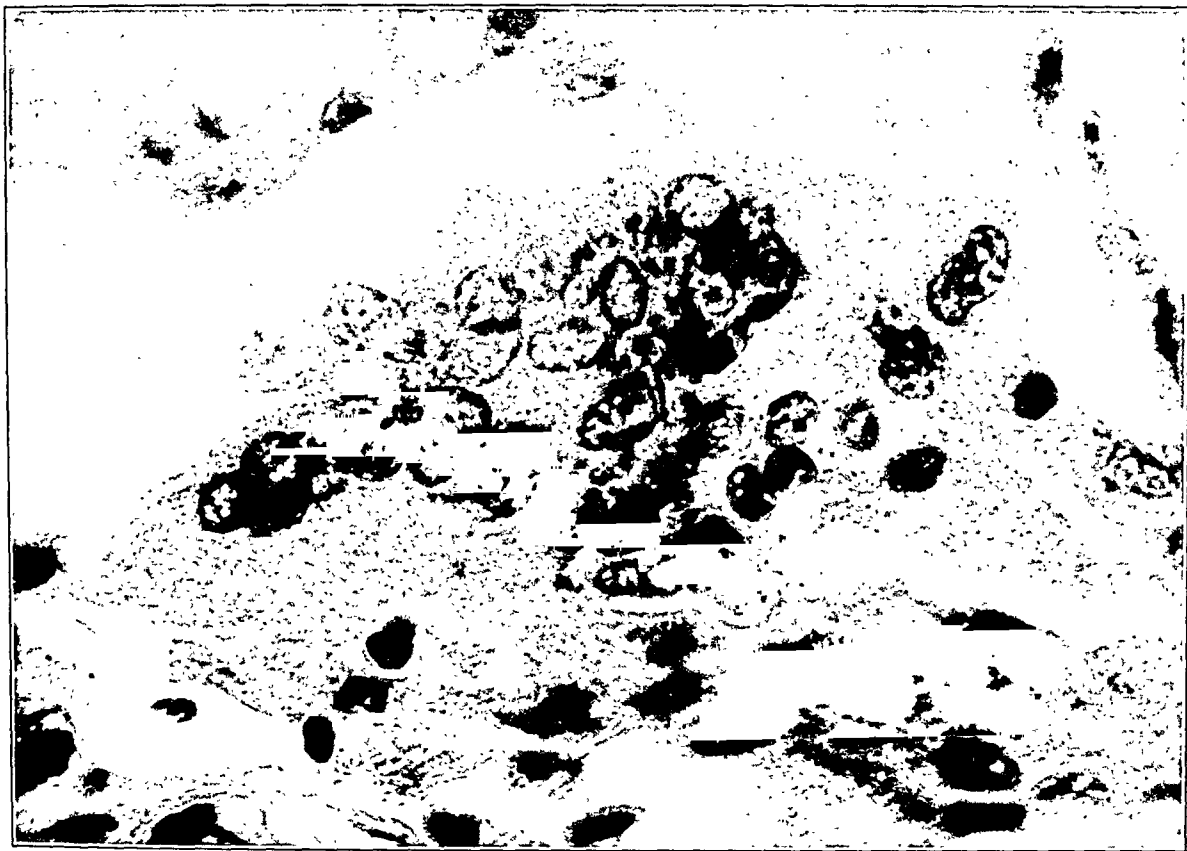


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Wright

Injection of Gases into Subcutaneous Tissues





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Wright

Injection of Gases into Subcutaneous Tissues

It was noted also that the medulloblastomas were very sensitive to radiation and subsequent experience has been consistently favorable as described by Bailey, Sosman, and van Dessel.<sup>8</sup> Similar results have been obtained by Olivecrona and Lysholm. Yet in spite of a radical surgical extirpation followed by subsequent radiation, a recurrence of symptoms with ultimate fatality has been the invariable rule. It is with the end-results in some of these more thoroughly treated cases that I wish to deal in this communication. The record of the first patient will be given briefly since it has already been recorded by Bailey, Sosman, and van Dessel (Case 4).

CASE 1. *Clinical History:* (Surg. No. 13981) J. H., aged 6 years, was admitted Feb. 9, 1921, referred by Dr. E. Koplik of New York City. He had a typical syndrome of a central cerebellar tumor and a suboccipital operation was performed Feb. 14, 1921, during which only the inferior portion of the tumor, which projected downward into the spinal canal, was removed. He was then given roentgen treatments with such a prompt and thorough improvement that treatment was stopped in May 1921, after only four treatments, and he remained well until March 1925.

When he was readmitted (Surg. No. 23390) on March 10, 1925, he had so few symptoms that it was decided to give him another roentgen treatment and let him go home. The treatment was given March 23rd. It was followed by vomiting, and gradually developing coma, so that by 5 A.M. March 24th, he was unconscious. An immediate suboccipital operation was performed. The bone had completely reformed over the cerebellar region. A soft, centrally placed tumor was found which was apparently removed *in toto*. He recovered promptly. The removal had been so complete that no roentgen treatment was advised.

His symptoms returned in September of the same year and he was readmitted (Surg. No. 25444) on Dec. 24, 1925, with bilateral choked discs and marked symptoms of cerebellar involvement. On Jan. 4, 1926 the suboccipital region was again explored. The whole right cerebellar fossa was filled with soft neoplastic tissue in which was a large cystic cavity. Because of his poor general condition an extirpation was not attempted until January 15th. It was not successful because of involvement of the bulbar nerves in the tumor. He was discharged February 16th, and died on May 31, 1926.

*Comment:* It was concerning this patient that Bailey and Cushing<sup>7</sup> expressed themselves so optimistically in 1926 following his second operation. In fact they were so confident that a complete extirpation had been accomplished that no postoperative radiation was advised, undoubtedly an error in judgment in view of the subsequent clinical course. That roentgen therapy is able to prevent a local recurrence for long periods of time is proved, among others, by the following case.

## FURTHER NOTES ON THE CEREBELLAR MEDULLOBLASTOMAS \* THE EFFECT OF ROENTGEN RADIATION

PERCIVAL BAILEY, M.D., PH.D.

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In 1925 I isolated, and described with Harvey Cushing,<sup>1</sup> a common type of midcerebellar glioma of childhood of which I found twenty-nine examples in Dr. Cushing's<sup>2</sup> collection. These tumors were very cellular, consistently rapid in evolution, and were situated almost invariably in the central part of the cerebellum just over the fourth ventricle. Tumors of similar type have since been recognized and reported by Olivecrona and Lysholm<sup>3</sup> and others, and I have observed thirty-four new cases up to June 1929.

These midcerebellar medulloblastomas are composed for the most part of carrot-shaped cells with delicate cytoplasmic extensions which form, among the nuclei, clear spaces known as pseudorosettes or ball-like spaces (*Markballen*), Wright.<sup>4</sup> Spongioblasts are numerous and I was inclined to call the tumors *spongioblastoma indifferendale* until I found that the neoplastic cells apparently differentiated also into neuroblasts, whereupon I coined the term *medulloblastoma*. This term indicates that the cells represent an embryonic stage earlier than either the spongioblast or neuroblast, a stage corresponding to the *indifferente Zellen* of Shaper, or *medulloblasts* as I prefer to call them. Neuroblasts had previously been identified in what appear to have been tumors of this type by Vanzetti,<sup>5</sup> and by Masson and Dreyfus.<sup>6</sup>

Experience with the surgical removal of these growths prior to 1925 had been very disappointing but was later more successful, and Bailey and Cushing<sup>7</sup> were led to express themselves more hopefully concerning their prognosis. They said in 1926 that they had had "one or two apparently successful extirpations . . . so that under favorable circumstances, in spite of its desperate situation, a more radical attempt at extirpation than has commonly been made in the past may be justifiable."

\* Received for publication October 4, 1929.

*Operation:* The operation was performed on May 7th, and disclosed an enormously dilated fourth ventricle filled with clear cerebrospinal fluid. The tectum was widely distended and in its depths could be seen the posterior commissure. The whole cavity formed a great cyst completely closed, except for the tectum, by a smooth, glistening wall. On the left side of the ventricle, where tumor had been left at the previous operation, was a flat yellowish gray nodule about half a centimeter in diameter. This nodule was removed. High up on the right side was another small mass of tumor which was destroyed by the electric cautery. The cyst wall could not be separated from the dura so as to allow access to the peripontine region.

The child was quite well until May 17th when he suddenly developed a hyperthermia. Repeated lumbar punctures, punctures of the fourth ventricle and of the lateral cerebral ventricles tided him over this period, but he subsequently declined and died Aug. 8, 1928.

*Microscopic Structure of the Tumor:* The neoplastic tissue removed at the first operation, as previously mentioned, showed the typical structure of a medulloblastoma, being composed of small cells with scanty delicate cytoplasm elongated at one extremity. The open spaces formed by the cytoplasmic extensions were very prominent (Fig. 1a) including numerous pseudorosettes. Spongio-blasts could be identified but no neuroblasts. Mitotic figures were numerous and almost invariably quite normal.

The fragment of tumor removed at the second operation differed widely in structure. The pseudorosettes were absent. Innumerable giant cells containing a dozen or more nuclei were seen everywhere (Fig. 1b). Mitoses were frequent, but rarely normal (Fig. 2). The tissue was greatly degenerated and contained much fat.

*Comment:* It is evident that the growth of the tumor in this case had been effectively checked by the radiation, the symptoms having been due to block of the circulation of cerebrospinal fluid by the cerebellum which was plastered everywhere to the wall of the sub-tentorial cavity in such a way that the obstruction could not be relieved at operation.

In several other cases there have been no signs of a local recurrence in the region of the cerebellum after a period of several months, but the patients have died from extension of the tumor elsewhere. It was for this reason that radiation was given to this patient over the entire spinal canal, in the hope that a possible intraspinal extension might be killed before it began to develop.

In the following two cases such an extension occurred and was subsequently treated by the roentgen rays with transitory improve-

CASE 2. *Clinical History:* (Surg. No. 30085) D. M., aged 5 years, was admitted Nov. 12, 1927, referred from the Children's Hospital, and complaining of dizziness and vomiting.

In August 1927, the child began to vomit without nausea, usually in the morning. In October he complained of dizziness and staggered when walking. Two weeks later his vision failed and he complained from time to time of pain in the back of his head.

He was admitted to the Children's Hospital where a suboccipital operation was performed by Dr. Horrax on November 7th. A midline cerebellar tumor was disclosed but no attempt was made to remove it because of the child's general feeble condition.

When admitted to the Peter Bent Brigham Hospital there was present marked hypotonicity of all the extremities with incoördination predominating in the legs, left internal strabismus, nystagmus on looking to right and left, and receding choked discs.

On Nov. 15, 1927, the suboccipital region was again opened, and a fairly complete extirpation was made of a typical medulloblastoma of the central cerebellar region, opening widely the fourth ventricle. A fringe of tumor was left on the left margin of the ventricle.

There was a slight postoperative hyperthermia after which the child recovered promptly and was discharged Dec. 11, 1927. At this time he was relieved of all subjective symptoms but was so incoördinate as to be unable to walk alone.

Microscopic examination of the tumor removed showed the typical structure of a medulloblastoma with numerous pseudorosettes (Fig. 1a). No neuroblasts were identified by Cajal's reduced-silver method. He was therefore given X-ray treatments as follows: Dec. 5, 1927, cerebellar area; Dec. 8, 1927, cerebellar area; Jan. 18, 1928, cerebellar area, cervical and upper dorsal spine; Jan. 25, 1928, lower dorsal and lumbar spine; Feb. 1, 1928, cerebellar area and upper dorsal spine; Feb. 8, 1928, lower spine; April 23, 1928, cerebellar area, cervical and upper dorsal spine; April 26, 1928, cerebellar area, cervical and upper dorsal spine; April 30, 1928, lumbar spine; May 6, 1928, lumbar spine. A target distance of 40 cm. was used, 4 ma., 182 kv.,  $\frac{1}{2}$  cu. and 1 al. filter, 30 minutes exposure, except for the treatments of April 26th, and May 6th when only 20 minutes were given. These treatments were followed by nausea and vomiting for two or three days.

He was examined on Jan. 18, 1928. The fundi showed marked secondary atrophy without choking. There was a slight left abducens palsy and slight nystagmus. The suboccipital region was not bulging. He walked alone but with a broad base. There had been no headaches nor vomiting. On February 15th his mother reported that he had been vomiting since his last treatment. He was much more unsteady in walking and the suboccipital region was full but not particularly tense. By April 11th he was having frequent headaches and vomiting and the suboccipital region was quite tense. Since the subsequent radiation produced no improvement he was readmitted to the hospital.

*Second Admission:* (Surg. No. 31181) At this time he was very incoördinate although still able to walk alone. There was a marked nystagmus and a suggestive Babinski-response on the left side. The suboccipital region was bulging but there was no choking of the optic discs. He was much emaciated and on the ward became increasingly apathetic. These symptoms, together with the headaches and vomiting, indicated a local recurrence for which an exploration was advised.



nerve fibers could be impregnated in the tumor and nothing resembling neuroblasts was found. Reticulin was scanty. There were very few pseudorosettes (Fig. 4a). The tumor was undoubtedly a medulloblastoma although more spindle-celled than usual. Mitoses were numerous.

The nodules on the surface of the spinal cord at autopsy, we were surprised to find, consisted almost exclusively of bands of collagenic tissue (Fig. 4b). Cells were rare; the nuclei pyknotic, and mitoses were infrequent and abnormal. Underlying the nodules of tumor there was an extensive gliosis. One area was found in which the tumor had actually invaded the substance of the cord (Fig. 6b). The roots of the spinal nerves caught in the nodules of tumor showed increase in connective tissue and degeneration of the myelin sheaths. The blood vessels were often thrombosed or occluded, apparently by pressure of the surrounding collagenic tissue aided by proliferation of the intima.

*Comment;* In those cases of extension of medulloblastoma into the spinal leptomeninges which have not been treated with the roentgen rays, there is much more connective tissue in the meningeal extension than in the original cerebellar tumor (*cf.* Fig. 5), but the tumor remains very cellular, and the connective tissue consists mainly of strands of reticulin. The extensive sclerosis of the spinal tumor in Case 3 must have been caused by the radiation.

It is unfortunate that the condition of the intracranial lesion could not be determined. Its condition may be imagined after the description of the following case, whose history is very similar to the one just recorded.

CASE 4. *Clinical History:* (Med. No. 28682) J. M. W., aged 18 years, was admitted Oct. 4, 1926, referred from the Outpatient Service. He was found to have a typical syndrome of a tumor in the central cerebellar region and was transferred to the surgical service (Surg. No. 27371) where a suboccipital operation was performed on Oct. 14, 1926, disclosing a midline tumor occupying the whole of the vermis. Beginning at its lower extremity it was apparently dissected out entirely, leaving the fourth ventricle widely exposed. He recovered promptly and was discharged on February 7th, completely relieved of his symptoms with the exception of slight hypotonicity of the extremities.

He returned later in February 1927, complaining of a girdle-pain in the right lower thoracic region, with numbness and weakness of both legs. There was hypoaesthesia extending up to the tenth thoracic segment, predominating in the left leg, and weakness and spasticity of both legs, especially of the right. There was no disturbance of the bladder but the paraplegia was sufficiently

ment. The condition of the spinal tumor when removed at autopsy was most interesting. The histories of these two patients have also been given by Bailey, Sosman, and van Dessel <sup>8</sup> (Cases 11 and 12), and were mentioned also by Cushing <sup>2</sup> (Cases 6 and 10).

CASE 3. *Clinical History:* (Surg. No. 23932) F. T., aged 11 years, was admitted on May 15, 1925, referred by Dr. V. A. Reed, of Lawrence, Mass., complaining of headaches and vomiting. The clinical history and findings were typical of a midcerebellar tumor and he was operated upon May 20th. The tumor, which weighed 31 gm. and projected downward through the foramen magnum, was removed fairly intact in one large mass. He recovered promptly and was given roentgen treatments over the cerebellum only, on June 12, July 16, Aug. 7, Aug. 28, Sept. 18, and Oct. 9, 1925, a full suberythema dose at each session.

When examined Dec. 28, 1925, he was perfectly well. There were no signs of cerebellar disturbance. He had been playing football and other games as though he had never been ill.

On October 12, 1926, he returned for examination because of pain in the chest and was readmitted to the hospital (Surg. No. 27429). There were no symptoms of disturbance in the cerebellar region, but there was definite loss of temperature-sense and hypoalgesia of the right side below the level of the third thoracic segment. The left leg was spastic, with exaggerated tendon-reflexes, clonus, defense-reflex and Babinski-response. The gait was very spastic and hemiplegic. There was occasional incontinence of urine. He was given a full suberythema dose of roentgen rays over the cerebellar, cervical and upper dorsal regions on November 4th. Other similar treatments were given Nov. 27, Dec. 20, 1926; Jan. 10, Jan. 31, Feb. 2, and Feb. 24, 1927. By March he was walking with scarcely discernible spasticity. The suboccipital region was flat and he was feeling quite well. By April still further improvement had occurred. Although the tendon reflexes were still exaggerated in the left leg, the strength was practically normal and he was running and playing baseball like a normal boy. No sensory defect could be found and the bladder functioned normally.

In spite of the improvement roentgen treatment was continued on March 18, and April 15. When he reported for treatment on May 11, 1927, he was pale and listless and complained of pain over the right eye. He was still almost free from spinal symptoms. The cerebellar region was treated. Following this he declined rapidly. Headaches set in about May 29; he became incontinent, drowsy, completely paraplegic, and died June 17, 1927.

*Autopsy:* The spinal cord was removed at the patient's home. Examination of the brain was refused. The spinal cord was covered by numerous flat, whitish nodules beneath the arachnoid, extending from the upper dorsal region to the cauda equina; they were mainly situated on the dorsal surface (Fig. 3).

*Microscopic Description:* The original tumor from the cerebellum was composed of small, closely packed cells having a small amount of cytoplasm extending from one extremity. Neither neuroglial nor

cavity occupied by the tumor had a fairly smooth wall but it was studded with several nodules of tumor and one nodule the size of a pea was perched upon the base of the fourth ventricle just at the posterior end of the pons. In the aqueduct of Sylvius and also throughout the lateral ventricle on the left side there were multiple nodules varying in size from 1 to 4 mm. in diameter. There was in addition a great mass of tumor which occupied the entire floor of the third ventricle and obscured the usual landmarks — chiasm, hypothalamic region and infundibulum. This mass of tumor measured about 2.5 cm. in its transverse and vertical diameters. It was whitish in appearance and looked somewhat different from the ventricular nodules. This extensive and widespread implantation of tumor throughout the ventricles did not seem to have obstructed the pathway of the cerebrospinal fluid anywhere, for the foramina of Munro were open and also the aqueduct. The foramen of Luschka was evidently open on either side. The ventricle showed only a moderate dilatation but the ependyma was everywhere studded with tumor nodules.

*Microscopic Description:* The tissue removed at operation had the typical structure of a medulloblastoma, with numerous pseudorosettes (Fig. 7a). There was very little reticulin and no neuroblasts were identified.

The spinal implants removed at autopsy consisted almost exclusively of masses of collagenic fibers with occasional small islands of neoplastic cells (Fig. 7b). Under the nodules of tumor, which lay mostly on the dorsal surface of the cord, was a most intense gliosis (Figs. 6a and 8b). Elsewhere the cord showed only a slight thickening of the pia mater and a slight peripheral gliosis (Fig. 8a).

The extension over the base of the brain contained much more numerous tumor cells and much less collagenic tissue. The nodules on the walls of the ventricles had the typical structure of the medulloblastoma. The ependyma had disappeared beneath them but there was only slight invasion of the underlying fibrous neuroglia.

*Comment:* In view of the spread of the tumor over the base of the brain as disclosed at autopsy it is unfortunate that no details could be obtained concerning the later course of his illness. The family was unobserving and I was unable to learn whether there had been polyuria or other symptoms of involvement of the hypothalamus.

advanced so that he was unable to walk alone. There were no signs of a return of the cerebellar lesion.

He was given on February 26th a roentgen treatment over the spine from third dorsal to second lumbar, which resulted in retention of urine for three days, but almost immediately improved the paraplegia. The roentgen treatment was repeated March 19th and April 13th. On March 23rd the cerebellar area was also treated.

By June 22, 1927 his condition was excellent. He was walking without assistance. The strength of his lower extremities was good but some spasticity remained. No sensory disturbance could be found, no disturbance of the function of the bladder and no signs of intracranial involvement. He complained of pain in the left shoulder but no sensory defect could be found in this region.

On July 5th the cerebellar area and cervical spine were treated, about half an erythema dose being given. He was next seen on December 7th, when he returned because of sudden weakness of the legs and bladder disturbance. A series of roentgen treatments was therefore given to the dorsal spine in 25 per cent doses on Dec. 7, Dec. 23, and Jan. 6, posteriorly, and Dec. 15 and 29 from in front. The bladder disturbance disappeared and he began to walk a little, but by February developed a paraplegia in flexion with severe cramps in the legs. He was given 50 per cent doses over the dorsal spine on Feb. 25 and March 2, and over the cerebellum on Feb. 6 and Feb. 9. There was no improvement. His vision slowly failed during the last two months of life without evidence of intracranial pressure. He died suddenly at home March 8, 1928. Permission was obtained for a postmortem examination.

*Autopsy:* The body was brought to the hospital for examination. The brain and spinal cord were removed, the brain having previously been injected with 10 per cent formalin *in situ* through the carotid arteries. General examination of the viscera revealed nothing of note.

The cerebellum was densely adherent in the suboccipital region where there were several walled-off pockets containing 20 to 25 cm. of xanthochromic fluid. Superficial examination of the brain revealed a dense whitish tissue covering the tuberian region and surrounding the carotid arteries. The hypophysis seemed slightly flattened. The leptomeninges seemed thickened and whitish also over the pons and the inferior surface of the left cerebellar hemisphere.

There were very dense arachnoidal adhesions all along the spinal cord which was covered by numerous flat, whitish nodules scattered from the cervical region to the cauda equina. The cord looked much like that of Case 3 (*cf.* Fig. 3).

On median section of the brain an amazing picture of multiple metastases within the ventricular spaces was disclosed. The old

true it seems to be advisable to radiate thoroughly the entire cerebrospinal system following an operation for one of these tumors in the hope of killing the scattered cells before they have time to implant themselves, for once they have become implanted and have begun to give rise to symptoms, a fatal outcome is inevitable.

The reason for the progression of symptoms when the tumor has been thoroughly checked and even almost completely destroyed is not at once apparent. It may be that the dying neoplastic cells release a toxin which is damaging to the nervous system, but from a study of my specimens it seems rather that the progression of symptoms is largely due to the transformation of the tumor into scar tissue with a resultant disturbance of circulation within the nervous system.

The roentgen rays are not supposed to injure the normal nervous tissues or meninges in the doses used. But the presence of the neoplastic cells in the meshes of the pia-arachnoid gives rise to a chronic irritation and proliferation of this tissue, in which condition it may be more sensitive to the radiation. At any rate it is evident that the radiation transforms the tumor-infiltrated leptomeninx into dense fibrous bands which constrict the cord and nerves, diminish the circulation of the blood and block the circulation of the cerebrospinal fluid.

Under these circumstances one might ask whether these patients have not been given too much roentgen treatment. Would it not be better to radiate thoroughly the entire cerebrospinal axis immediately after operation and then stop? Time and further experience alone can tell.

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It is possible that such an extension had occurred also in the previous case, but the brain could not be examined.

### DISCUSSION

Further experience with these medulloblastomas has not altered in any way the conception of their structure or clinical course detailed by Bailey and Cushing.<sup>1</sup> They are composed of very embryonic undifferentiated cells of neuroepithelial origin and grow with extreme rapidity. It is doubtless for this reason that they are so sensitive to radiation.

It has been proved by Bailey, Sosman, and van Dessel,<sup>8</sup> and confirmed by Olivecrona and Lysholm,<sup>3</sup> that the growth of these tumors may be checked for a considerable length of time by roentgen therapy and yet a fatal outcome has ensued in every case. It is generally assumed that after a certain time the neoplastic cells become radioresistant, but in view of the cases here reported it is legitimate to ask if this is really the correct interpretation.

In Case 1 it may be considered that roentgen treatment was not given a fair trial since none was given after the main body of the tumor was removed, but in subsequent cases the cerebellum was persistently radiated and in no instance where the main mass of the tumor was removed and the iter definitely freed had there been signs of a local recurrence except in Case 2 here reported, in which it was proved at operation that the return of symptoms was not due to growth of the tumor. It was proved also in Case 4 at autopsy that there had not been a local recurrence.

The patients in whom a fairly complete enucleation was made and who were treated intensively with the roentgen rays nevertheless died from an intraspinal or intracranial extension of the tumor. Of course such an extension may occur without operation, as is amply proved by my own experience and the cases recorded by others, but its occurrence after operation is so frequent as to preclude the simple development of an extension already present at the time of operation. The neoplastic cells must be scattered into the cerebrospinal spaces by the operator, falling by gravity into the spinal canal or being carried upward by the current of fluid over the base of the brain, where they grow as implantation metastases. This being

## DESCRIPTION OF PLATES

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### PLATE 26

- FIG. 1. CASE 2. (a) Tumor removed at first operation containing numerous pseudorosettes. Methylene blue and eosin.  $\times 300$ .  
(b) Tumor removed after radiation. Note the numerous multinucleated cells. Hematoxylin and eosin.  $\times 300$ .
- FIG. 2. Case 2. Types of abnormal mitoses after radiation. Note the giant cell in the middle photograph. Methylene blue and eosin.  $\times 1200$ .

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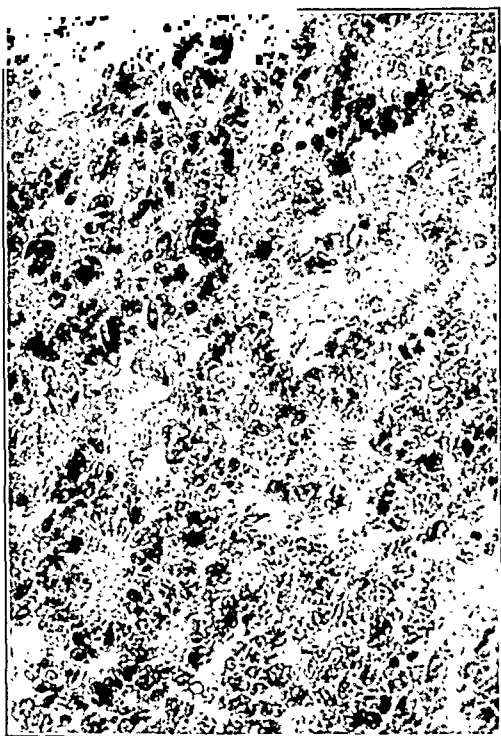


PLATE 27

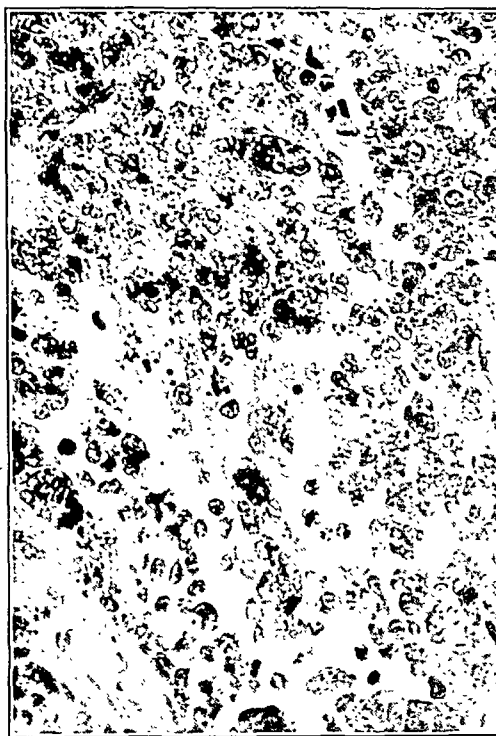
FIG. 3. Case 3. Dorsal surface of spinal cord showing nodules of tumor.

FIG. 4. Case 3. (a) Tumor removed from cerebellum. Hematoxylin and eosin.  
× 300.

(b) Tumor removed at autopsy from surface of spinal cord.  
Note the sclerosis probably produced by radiation.  
Methylene blue and eosin. × 100.



a



b

1

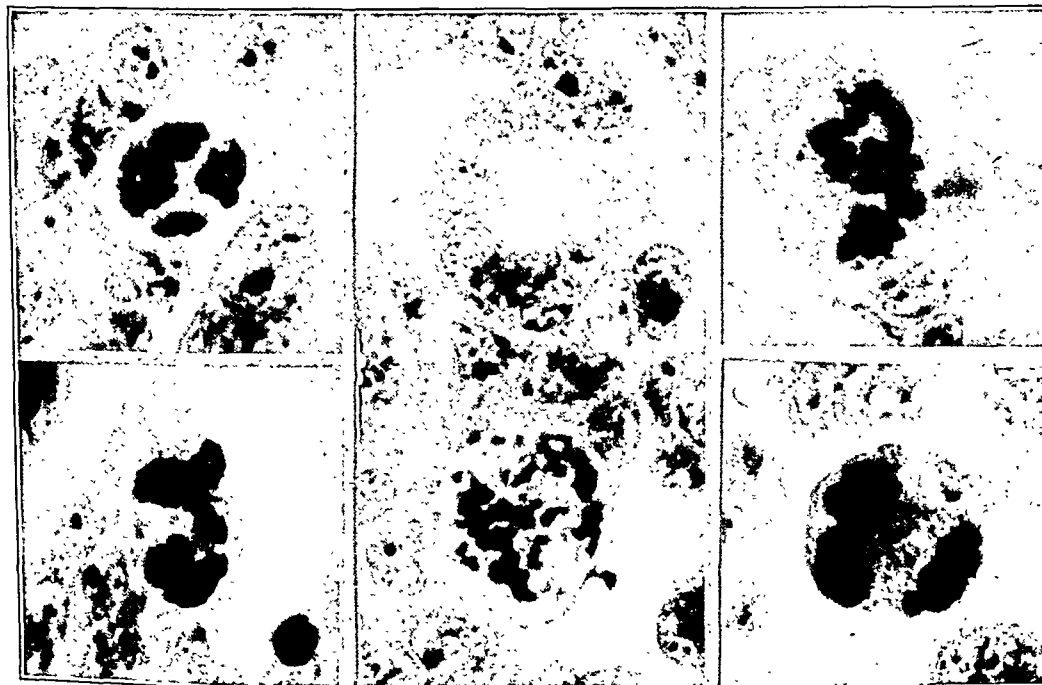


PLATE 28

FIG. 5. (a) Medulloblastoma from cerebellum. Hematoxylin and eosin.  $\times 300$ .

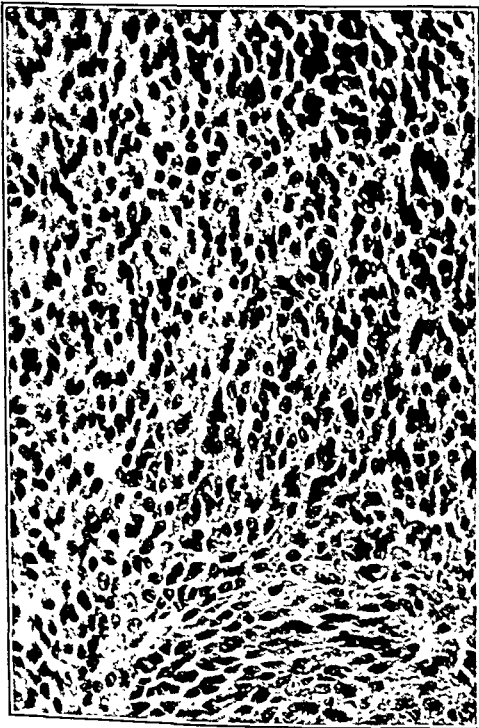
(b) Spontaneous extension to the spinal meninges, untreated by radiation. Note the excess of connective tissue. Hematoxylin and eosin.  $\times 300$ .

FIG. 6. (a) Case 4. Sclerosed tumor on dorsal surface of lower cervical cord with extensive underlying gliosis of posterior columns. Phosphotungstic acid hematoxylin.  $\times 5.5$ .

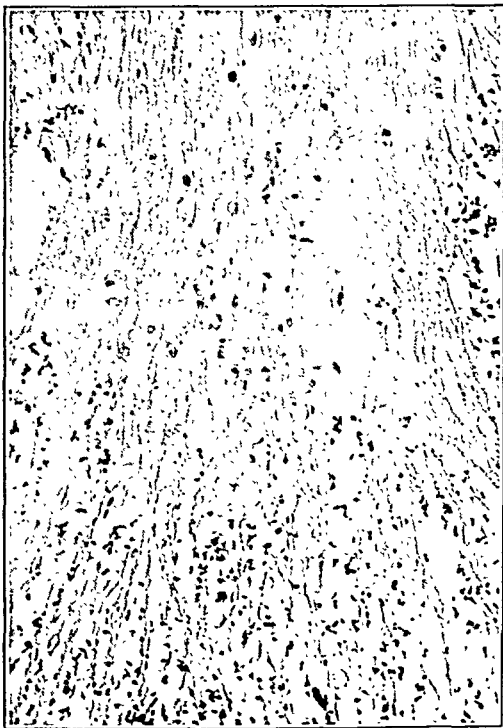
(b) Case 3. Sclerosed tumor on lateral surface of thoracic cord, invading the posterior horn. Note the surrounding gliosis. Phosphotungstic acid hematoxylin.  $\times 5.5$ .



3



a



b

4

PLATE 29

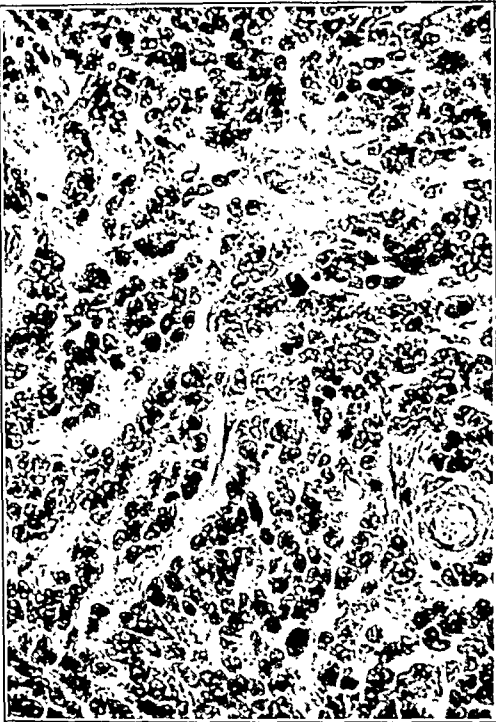
FIG. 7. Case 4. (a) Tumor from cerebellum. Methylene blue and eosin.  $\times 300$ .

(b) Spinal tumor after radiation. Note the intense sclerosis and one island of persisting neoplastic cells. Hematoxylin and eosin.  $\times 300$ .

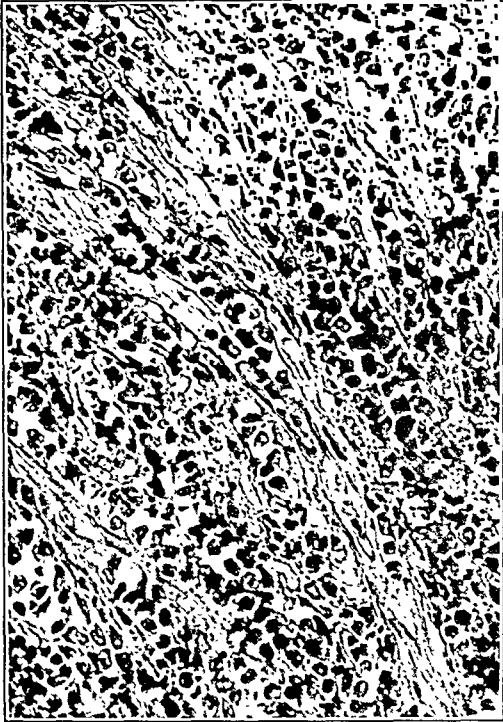
FIG. 8. Case 4. Photographs from periphery of spinal cord. Phosphotungstic acid hematoxylin.  $\times 300$ .

(a) Area not covered by tumor. Note thickening of pia mater and slight marginal gliosis.

(b) Area covered by nodule of tumor. Severe gliosis extending deeply into the cord.

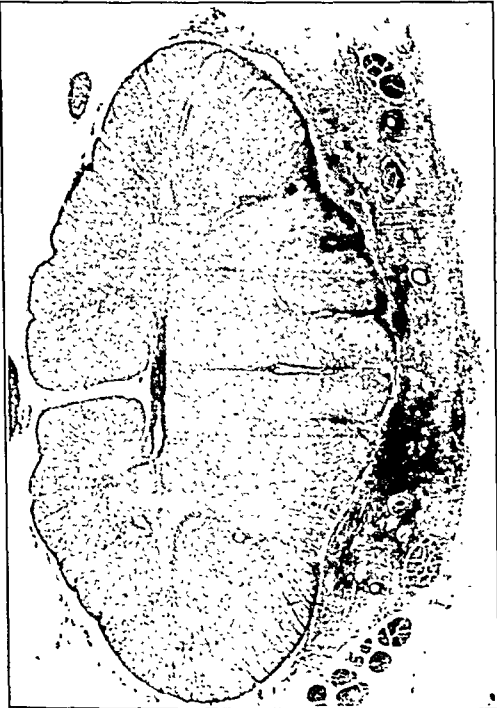


a



b

5



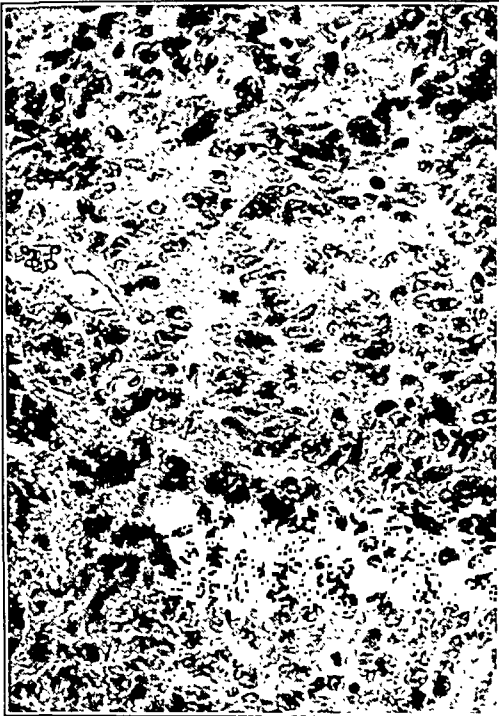
a



b

6





a



b

7



a



b

8



of softer tissue elements, all enclosed in a tough fibrous tissue capsule.

*Microscopic Description:* The tumor is well encapsulated by dense connective tissue. The stroma consists of mucoid connective tissue with much cartilage. Here and there aggregates of fat cells are noted. Groups of epithelial cells, oval, ovoid or round in shape are scattered throughout, together with definite tubular structures lined with two or more layers of cuboidal cells. Some few such present a single lining layer of low columnar cells. Typical squamous epithelium with rudimentary "pearl" formation is noted.

CASE 2. A.P. (C.H.-Cl. 6), colored female, 43 years of age, service of Dr. Cox, has noticed a tumor on the right side of her palate for the past six years. Is painless, but recently has grown until it now extends beyond the middle line, causing some interference with mastication and deglutition. General health excellent.

#### PATHOLOGICAL EXAMINATION

*Gross Description:* The specimen (S-29-2874) consists of an oval tumor mass measuring 4 by 2.5 by 3 cm. and sectioned in its longest diameter. Externally it presents an irregular reddish brown color, except for the surface of attachment which is grayish in color. The mass is well encapsulated, firm and resistant to touch. The interior revealed by sectioning shows smooth, creamy white surface, with here and there darker areas of brownish red and reddish gray. Pin-head and somewhat larger yellowish brown and generally softer areas are studded throughout, suggestive of necrosis. The mass cuts smoothly but some resistance is encountered on sectioning. Arborizations of dense fibrous tissue are encountered at the base, but not throughout the tumor substance.

*Microscopic Description:* In general the histology is rather similar to that noted in Case 1 but glandular formation is not as well marked, most of the tubular structures being rather rudimentary. There is marked merging of the epithelial elements into the mucocartilaginous stroma, by means of both cellular projections and intercellular fibrillae. Keratinization is nowhere noted. The connective tissue capsule, rather dense in structure, is invaded by aggregates of epithelial cells and rudimentary tubular structures.

## MIXED TUMORS OF THE PALATE \*

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The much debated and to some investigators entirely inappropriate term, "mixed tumor" as applied to the clinically characteristic and histologically complex neoplasms occurring in the general oral-facial region, will undoubtedly remain a descriptive term in the nomenclature of pathological anatomy, and so it should. Admittedly conveying no idea of the source and mode of development of these newgrowths, it has the value of conveying the thought of their actual complexity of composition and, at times, of structure.

Mixed tumors occur rather frequently in the salivary glands and comprise the typical neoplasm of the parotid. Similar tumors occur on the cheeks, lips, nose, gums and palate, the latter growths being the most common with the exception of those of the parotid and submaxillary glands. Even in spite of this relative frequency, scarcely 100 mixed tumors of the palate have been described. This fact prompted the present report of two cases recently seen.

CASE 1. *Clinical History:* Mrs. M.S. (C.H.-G. 9245-No. 302), service of Dr. Taquino, white, female, 25 years of age, admitted to the hospital for tumor of palate. Past and familial histories of no importance. About one year ago noticed a small tumor on the left side of her palate which has gradually grown until it is now the size of a hen's egg. Has experienced no pain, but some discomfort on talking and swallowing. Has lost no weight. Physical examination and laboratory tests negative. Clinical diagnosis, fibroma of the soft palate, left side. Under local anesthesia tumor was removed by enucleation.

### PATHOLOGICAL EXAMINATION

*Gross Description:* The specimen (S-29-1235) consists of a small, firm, whitish pink, tumor mass, irregular, but somewhat ovoid in shape measuring 3.5 cm. in its longest diameter and weighing 90 gm. It appears well encapsulated. On sectioning, the knife encounters much resistance, with glistening, grayish pink, reddish brown, and grayish white, surfaces revealed. Apparently the tumor is composed of cartilage, dense and loose connective tissue with some areas

\* Received for publication September 26, 1929.

tumor even passes imperceptibly into the normal glandular element giving the impression of its apparent derivation therefrom.

Much conjecture as to the nature and derivation of the various cellular elements of mixed tumors has existed. To some they are endothelial growths with derivation from the endothelium of tissue spaces and lymph channels; to others they are purely epithelial. This latter view is now generally accepted even though Volkmann, Steinhaus, Martin, von Hanseemann and Borst have endorsed and defended the theory of their endothelial nature. Even rather full acceptance of the epithelial nature of the parenchymal cells of mixed tumors as advocated by Humberg, Ribbert, Chevassu, Fock, Krompecher, and others has not fully quieted the arguments concerning them. There remained explanation of the mode of transition from ectodermal to mesenchymal elements. For this actual metaplasia is the explanation most generally offered. Even this explanatory theory appears cloaked under various forms. To Ehrlich, the metaplasia is principally due to secretion of seromucous material by the parenchymal cells. Marchand advances rather similar views. To him the stroma is purely epithelial in character. Böttner assigns little importance to the mesodermal elements. He believes the tumors uniform epithelial neoplasms and argues that even in the tumors in which cartilage and mucoid connective tissue are predominant elements they must be considered as tumors in which recession of the epithelial structures has taken place. Krompecher and Forman and Warren believe that there occurs an actual metaplasia of epithelium stroma, and advance arguments based on embryonal as well as morphological grounds in support of their position.

To Fraser, Masson and Peyron, Desmarest and Masson, the metaplastic processes accounting for the histology of mixed tumors are even more complex. They believe that the parenchymal cells come from normal glands, mucosal in the case of palatal growths, and that the mucoid connective tissue cartilage matrix arises from the epithelium of the tumor itself.

While metaplastic processes appear as essential to a full explanation of tissue transitions within a same class, they need not be resorted to for full explanation of all mixed tumors, nor even of any, if the broadest conception of embryonal sequestrations is accepted; for then, the number and character of sequestered tissue elements readily account for the varying histological character of the adult

## FREQUENCY OF MIXED TUMORS OF THE PALATE

Eggers, after a very careful survey of the literature and a painstaking and exhaustive study of pathological material, gross and microscopic descriptions and photographs of practically all mixed tumors of the palate reported, concludes that sufficient evidence exists to establish only 87 of the over 100 reported cases as authentic mixed tumors. To this list he adds 5 of his own. If he has erred in his inclusions it has been rather on the side of safety and his compilation of mixed palatal tumors can be accepted as the most accurate extant.

## STRUCTURE AND DERIVATION

Although some pathologists still believe that mixed tumors usually originate in fully developed glands, even several decades after birth, it is now generally conceded that there exists a developmental relationship between them and embryonal disturbances in the nature of cell displacements or enclavements. They should rather be regarded as tumors in, but not of, the structures they invade.

The histological structure of mixed tumors is most irregular. Generally they may be said to consist of a ground tissue of myxomatous material or cartilage with transitions of one into the other, and fibrillar connective tissue, with occasionally some little lymphoid tissue or bone interspersed. Within this basic matrix, cell inclusions, varying in individual cases and presenting no regularity as to structure, are present. Sometimes these parenchymal cells appear closely packed; at other times they are arranged as networks, strands or branching columns. In some tumors they simulate gland-like or tubular structures lined with cuboid or cylindrical cells. Again they may appear as irregularly shaped, large masses presenting no definite morphology, the individual cells ("indifferent cells") themselves being small or large, oval, ovoid, rounded or even spindle-shaped. Occasionally they present a whorl-like arrangement simulating the epithelial pearls of squamous celled neoplasms. Generally, these cells present an infiltrative type of growth, usually merging into the cartilage or myxomatous tissue masses without any sharp lines of delineation. This blending of the various tissue elements is always a characteristic feature. At times the parenchyma of the

## CONCLUSIONS

1. The term mixed tumor as applied to the neoplasms occurring in the general oral-facial region is distinctive and descriptive and should be retained. The origin of these tumors can be most satisfactorily explained by the theory of embryonal enclavement.
2. Microscopically complex but clinically benign, it is doubtful if typical mixed tumors ever undergo so-called malignant changes. Certainly such transformations, if occurring, are difficult of proof.
3. Palatal mixed tumors show the same general histological complexity and clinical characteristics as those occurring in other regions.
4. Two mixed tumors of the palate are reported.

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tumors. That the enclavement theory presents the correct solution of the problem of mixed tumor genesis appears at this time incontrovertible.

### MALIGNANCY OF MIXED TUMORS

Mixed tumors, wherever located, are, as a rule, nodular, firm, well encapsulated, rather freely movable and grow slowly. Although microscopically they may bear all the stigmas of malignancy, clinically they are said to "become malignant" when as a result, principally of trauma as thought by some, or for other reasons, they break through their enveloping capsule, begin to grow rapidly, cease to be movable, infiltrate their immediate vicinity, recur after removal and present metastasis.

In mixed palatal tumors, malignancy, as clinically understood, is most infrequent. In the 92 cases compiled by Eggers only 2 show histories of invasive or recurrent growths, but as Eggers rightly points out, most of the reports made concerning them were shortly after their removal. Again, the very location of these tumors and the discomfort they produce speak for their early removal before malignant metamorphosis, if such be possible, can eventuate. McFarland, after exhaustive study of 90 mixed tumors followed over a period of years, concludes that "malignant changes whether sarcomatous or carcinomatous in mixed tumors must be rare."

This conclusion is undoubtedly valid. Microscopic malignancy means nothing. "Recurrences" after operation must be carefully evaluated. Many of these tumors present technical difficulties to their complete removal and many "recurrences" are undoubtedly continuity of growth after incomplete removal, just as many "metastases" are simple extensions of growth.

### AGE AND SEX DISTRIBUTION

Statistics concerning mixed palatal tumors are not sufficiently comprehensive to allow accurate classification as to age and sex distribution. They have occurred at all ages, probably being more common in the second, third and fourth decades of life. There seems to be no sex predilection.

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## DESCRIPTION OF PLATES

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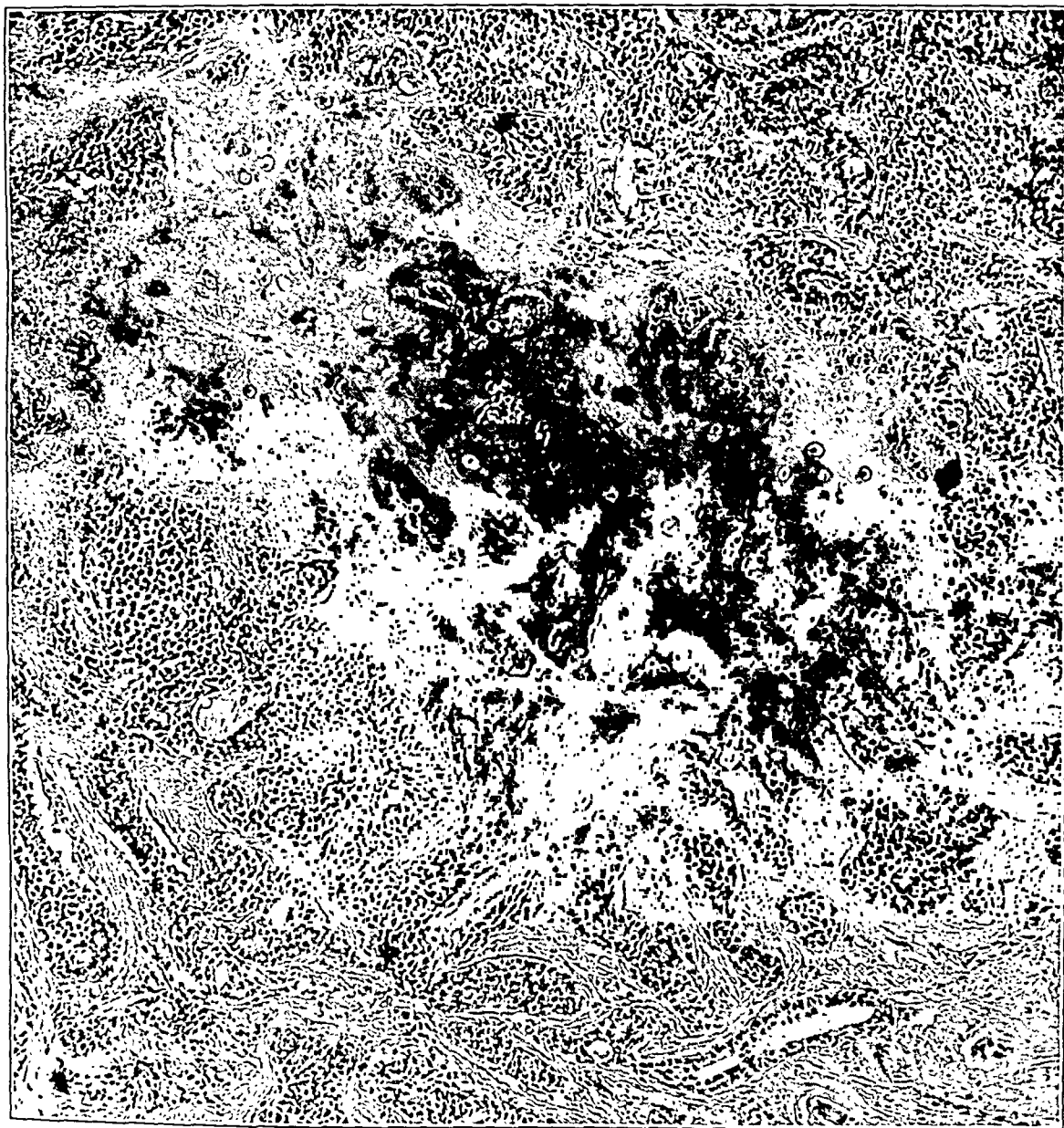
### PLATE 30

FIG. 1. Case 1. Glandular and squamous epithelium. Cartilaginous and myxomatous stroma.

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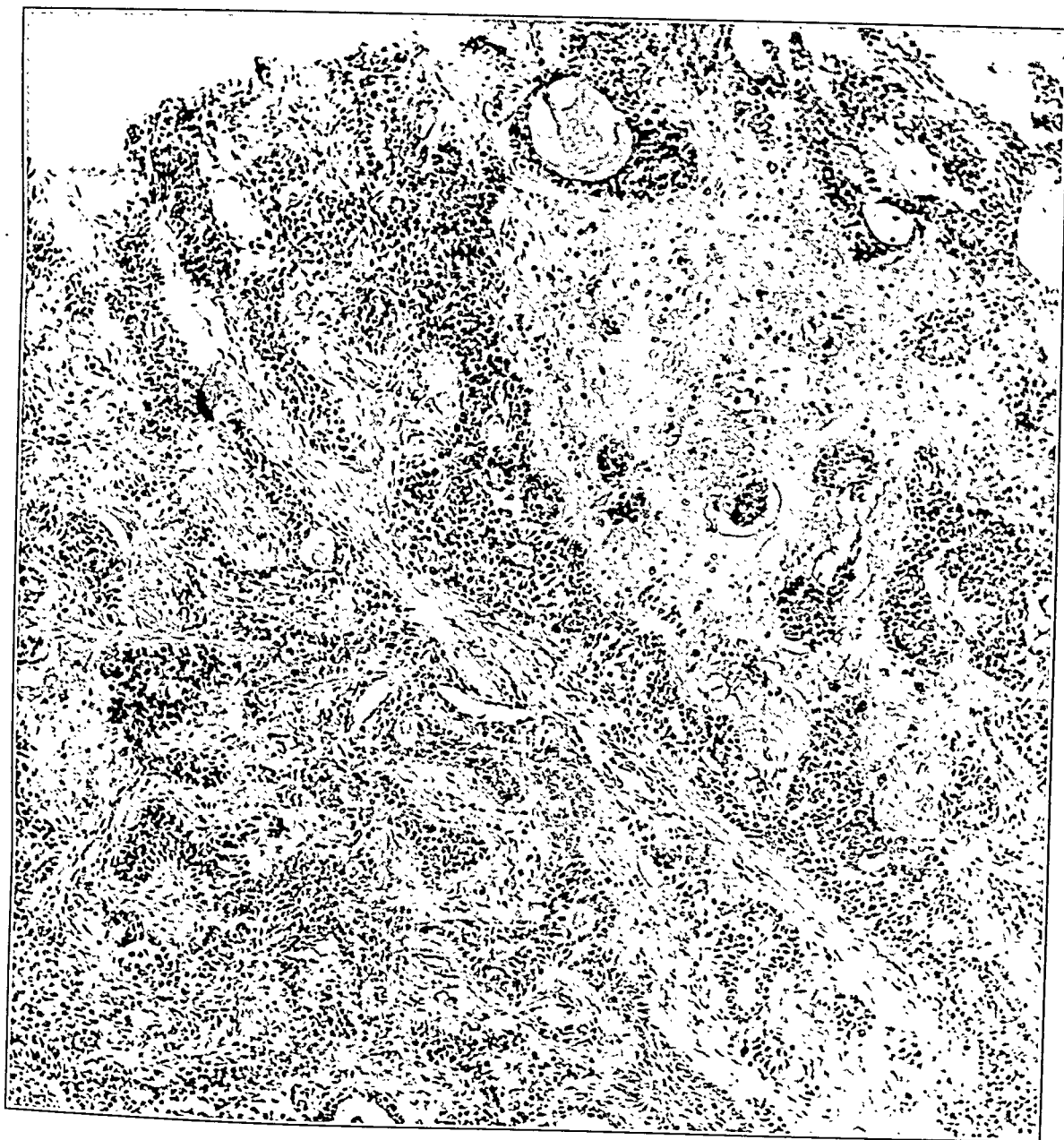
PLATE 31

FIG. 2. Case 2. Marked blending of epithelium and stroma.



I





The localization of amyloid confined to single foci is considered analogous to the development in scattered foci of degenerative changes in general, which, as Ribbert has advocated, are related to localized metabolic changes. Beneke and Bönning further suggest that the patchy distribution in the heart is no more remarkable than the generally known distribution of amyloid in other organs. With these cases of general amyloidosis the present study is not immediately concerned.

By far the less important group numerically, and, because of their uniqueness, perhaps unduly emphasized, are those cases in which the amyloid infiltrations are confined primarily to the heart, almost to the exclusion of those organs and tissues in which it is deposited in the usual cases of generalized or local amyloidosis. In this group there are, to our knowledge, only three reported cases at present.

In the case reported by Wild<sup>3</sup> there was found marked amyloid deposit in the myocardium associated with similar local deposits in the lungs, tongue, bladder and gastro-intestinal tract. The clinical report of this case is incomplete. The condition in the heart existed without true clinical signs referable to its existence. The patient, a 56 year-old woman who died of erysipelas had shown only the general clinical picture of heart failure. At autopsy the heart was small. Its walls were generally involved by nodular deposits of cartilaginous consistency, especially in the left auricle, and least pronounced in the left ventricle. Even the valve leaflets were infiltrated and thickened. There was an associated nodular infiltration of the peritoneum and intestinal serosa. According to Wild's observations the homogeneous masses which he found in the heart and intestine possessed a central zone which gave the amyloid reaction, and a non-specific hyaline peripheral zone.

The case of Steinhäus<sup>4</sup> presented changes remarkably similar to the findings of Wild. In a previously healthy 40 year-old man, the clinical syndrome consisted of vomiting, distention and intestinal hemorrhage, secondary anemia and clinical heart failure, the entire duration being but six months. Clinical observations on this case indicate a copious bleeding from the gastro-intestinal tract in a patient previously in good health, followed by marked anemia of the secondary type. The pulse was rapid. Albumin and traces of blood were found in the urine. In this case also there was apparent a stiff knot-like infiltration of the entire heart wall, stomach and

# A PATHOLOGICAL STUDY OF PRIMARY MYOCARDIAL AMYLOIDOSIS \*

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There have been reported two distinct groups of cases in which amyloid infiltration of the heart has been observed.

Within the first, and by far the more prominent group numerically, is a series of cases such as those reported by Von Huebschmann,<sup>1</sup> in which amyloid has been demonstrated in the hearts of patients affected by a generalized amyloidosis. In eight such cases investigated postmortem by Von Huebschmann only on microscopic investigation was amyloid demonstrable within the myocardium, and in no case was its occurrence in this location associated with specific differential symptoms referable to the system involved. Microscopically, the myocardium was found to contain amyloid deposits within the connective tissue and vessel walls; rarely, it was demonstrable in the valves and endocardium. In no instance was amyloid degeneration of the muscle fibers observed. On the basis of his observations, this investigator concluded that the amyloid arises both by transformation of the connective tissue fibrils (into amyloid) as well as by an interpositional deposit.

Commenting on this series in its review, Beneke and Bönning<sup>2</sup> suggest that Von Huebschmann's observations are in agreement with all similar reported cases. But they advocate that in primary myocardial amyloidosis the peculiar nodular amyloid deposits in the heart are not only confined to the capillary walls, but are localized about the muscle fibers themselves. It is their opinion that the accumulation of amyloid in the ectoplasmic zone of individual tissue cells leads to complete cellular intubation by amyloid, and that cells in this manner deprived of their nutrition undergo inanition atrophy. This they believe to be indicative of a previously unrecognized causal relationship between the metabolic processes of the sarcolemma-free muscle fibers, and the local deposition of amyloid.

\* Received for publication October 28, 1929.



On March 19, 1929, the patient came into the hospital with a second bad break in compensation. With salyrgan and theocin he lost a huge amount of edema and became compensated. His mental befuddling was maintained, however. He was incontinent of urine and feces, and developed bed sores. On March 29th he developed a chill and high temperature. On March 30th he was in deep coma with stertorous breathing, neck rigid, and questionable Kernig. A spinal puncture gave a cloudy fluid with a fibrin coagulum and 2000 cells. Culture of this fluid and blood culture showed a hemolytic streptococcus. He died March 31, 1929, in coma.

From the autopsy the following diagnoses were made:

Septicemia, streptococcus hemolyticus, blood culture; meningitis, acute, diffuse; pericarditis, acute, purulent, with purulent effusion; cardiac dilation and hypertrophy; myocardial necrosis, acute focal with auricular thrombus, puriform; amyloid deposits, myocardial, pulmonary and renal; nephritis, chronic vascular; congestion, spleen, liver, adrenal and renal; decubitus ulcers.

### HEART

The following description of the heart was given at autopsy:

The heart is a typical *cor bovinum*, weighing 700 gm. It is rubbery to compression, so that the depressed wall gives a sense of rebounding when the tension is released. The cut surface throughout is of a homogeneous reddish appearance as of stained liquid albumin, and gives one the impression that the light is reflected from the surface which is partially composed of a translucent substance. There is a puriform thrombus in the apex of the right auricle. The left auricle is clear to the limits of the appendage. The right auricular cavity is markedly dilated as is the left, and the walls of both are thickened to measure 0.5 cm. in diameter. The right ventricular cavity, it is estimated, is twice the normal size. The myocardium is 1 cm. thick at the base, and 3 mm. thick at the apex. The myocardium of the left ventricle is tremendously hypertrophied and the cavity increased to an estimated one-half greater than normal. At the base the wall measures 4 cm.; at the apex 2.75 cm. There are no grossly apparent necroses. The papillary muscles of both ventricles are markedly hypertrophied, some in the left ventricle measuring as much as 1.5 cm. in diameter at the base. The valves are free and flexible throughout. There are no vegetations. The mitral, aortic, tricuspid and pulmonic valves measure respectively: 11, 8.5, 8, and

intestinal tract. The cut surface of the heart was hard, of a glassy homogeneity and appeared whitish gray as if cooked. In the markedly thickened stomach wall were many glassy nodules, and numerous erosions of the mucosa. In this case, too, the liver, spleen and kidneys were free of amyloid. The amyloid deposits adjoined extensive deposits of hyaline-like material in the connective tissue. Often, particularly when in the vessel walls, those nodules gave the amyloid reaction. The staining reactions have been questioned by Beneke and Bönning, although they admit that the striking similarity to the case reported by Wild favors the correctness of the dye tests.

The case of Beneke and Bönning was a 70 year-old man who entered the hospital moribund with a diagnosis of chronic bronchitis. The amyloid deposits were limited almost exclusively to the heart, the adjoining vena cava and the lungs. Because it has been reported fully and accurately this case will be referred to in the discussion of the following one which fell under our observation, a case which has points of similarity to all of the foregoing, but differs from each of them. Our case follows:

### REPORT OF CASE

*Clinical History:* Feb. 8, 1926, a 65 year-old negro male, entered the hospital with a respiratory infection for which he was successfully treated. The essential findings at this time were: a generalized arteriosclerosis, normal heart, absent or sluggish reflexes, negative urine. Non-protein nitrogen 33.3, Wassermann and Kahn negative, sputum negative, stools negative, globulin cerebrospinal fluid, negative.

April 16, 1928, the patient was seen in the Out-Patient department complaining of cough of one months duration associated with shortness of breath on exertion and epigastric tenderness. Digitalis therapy was instituted.

Nov. 19, 1928, the patient entered the hospital with a typical history of progressive left-sided heart failure.

*Laboratory Findings:* Electrocardiograms: Nov. 19, 1928, auricular fibrillation, left ventricular preponderance. Dec. 13, 1928, auricular fibrillation, ventricular premature contraction. Dec. 14, 1928, auricular fibrillation, left ventricular preponderance. Dec. 15, 1928, auricular fibrillation, left ventricular preponderance, low voltage, inverted T in all leads.

He was discharged from the hospital Oct. 20, 1928, in a state of complete compensation. He had been diagnosed: general arteriosclerosis, arteriosclerosis of coronary arteries, cardiac arrhythmia-auricular fibrillation, cardiac insufficiency. He was sent out on digitalis 0.1 gm., and given instructions regarding diet, exercise and fluids. He returned to the Out-Patient department about two months later (Jan. 14, 1929) with slight dyspnoea and moderate edema. Digitalis was increased to 0.1 gm. b. i. d. and patient instructed as to conduct.

*Left Auricle:* In a tangential section of the left auricle the amyloid deposits have the characteristic tigroid appearance so apparent in the ventricles. In a cross-section they appear as minute, sometimes interlacing islets separated by the elements of the myocardium, endocardium, or epicardium.

### MICROSCOPIC EXAMINATION

*Myocardium:* The muscle cells throughout the entire myocardium are hypertrophic, a condition which is especially marked within the wall of the left ventricle. An occasional minute scar is apparent, especially in sections from the posterior portion of the left ventricular apex. The adjacent muscle cells are markedly enlarged. An estimated 50 per cent of the volume of the myocardial sections is represented by amyloid, which, throughout the myocardium is present in the crevices between the muscle bands and their composite fibers, and intracellularly. It is homogeneous, and is present in the form of eccentric laminated bands which in many instances give to this foreign element a whorl-like appearance. There are areas in which there is so great an abundance of this material that the muscle elements are entirely wanting or occur only as vestiges of an original cell or cells in a field composed almost entirely of amyloid. Not every bundle or fiber is equally affected, for while amyloid is found in the interstitial space between practically every muscle fiber, thus giving it a diffuse distribution, its deposition is not at all uniform. At times it is present as a narrow band which forms a sheath at one side of an interfibrillar capillary, not encroaching on the adjacent muscle fibers. In other instances large areas composed only of amyloid are present. Marked indentation of the myocardial cells by amyloid is readily apparent, but in a few instances only is the integrity of the muscle cell wall interrupted by these apparent invaginations. In serial sections these invaginations can be readily traced from simple indentations of the cell wall to actual cellular invasion so that one finds in examination of a single fiber serially the transition from simple cellular indentations by amyloid to actual intracellular deposits of this substance. When the amyloid present is actually intracellular, having gained entrance to the cell in this manner, the amyloid substance is often surrounded by a clear zone of sarcoplasm, the nucleus displaced and the myo-

15 cm. The endocardium throughout is clear and smooth with the exception of the right auricle which has already been described. The coronary arteries are moderately sclerotic. A small atheroma is present in the orifice of the left. It is recent. The coronary sinus is markedly dilated.

Because amyloid was suspected from the appearance of the heart, tissues were taken from every portion of this organ inclusive of pulmonary arteries, aorta, inferior vena cava, pulmonary veins, and pericardium. These tissues were rapidly stained in a Gram's iodine solution and transferred at once to sulphuric acid. The following tissues gave a positive reaction almost immediately.

*Left Ventricle:* A section of the muscle of the left ventricle with a specific amyloid stain, examined under the dissecting microscope, reveals an extensive bluish mottling of the tissue. These bluish areas are diffusely scattered throughout the entire section, running parallel to the line of fiber of the myocardium. In this manner the specimen is given a stippled or tigroid appearance with the stippling occurring in interrupted parallel bands. These bands are not complete in themselves, but have points of continuity with one another alternating with points of interruption. It is therefore evident that the deposit of amyloid is not uniform but diffuse throughout the myocardium. This observation has been recorded photographically by reflected light. The amyloid appears black in the reproductions. The appearance of the amyloid in the epicardium and beneath the endocardium of this ventricle is striking. Interlacing clusters of bluish material often related to definite vessels would by this stain indicate that, in the epicardium at least, the amyloid deposits have a definite relation to some portion of the vascular bed.

*Right Ventricle:* Within the right ventricle deposits are essentially the same as those in the left, and practically as abundant.

*Right Auricle:* In a section taken through the right auricle to include the flap of the foramen ovale, the amyloid has practically the same anatomical distribution as in the ventricles. Wherever the muscle fibers have been cut longitudinally in this section the same tigroid appearance is observed as was apparent in the ventricular myocardium. Because of the line of muscle fiber in this section, however, many of the deposits appear as minute interlacing islands in the photograph. The focal areas of endocardial infiltration of the diffuse type are again apparent.

especially medialward in this tissue. It has approximately the same relationship as that present in the para-aortic veins.

*Lungs:* Irregular intramural amyloid deposits are present generally throughout the alveolar walls of both lungs. The finer examination of these deposits shows that they are extensive, nearly diffuse about the alveolar capillaries where, at times it is so abundant as to cause the alveolar epithelium to bulge into the acinar space. The same transitions in the loss of the normal stromal connective tissue relationship between the capillaries and of the respiratory epithelium of the lungs is here observed as was so apparent in the interfiber deposits of the myocardium.

*Kidneys:* The renal arteries of medium size present a definite thickening of their intima, and in some instances actual occlusion of the lumen is apparent. In association with these vascular changes there occur diffusely throughout the cortex numerous triangular scars containing atrophic renal structures with large numbers of hyalinized glomeruli. The scars are infiltrated with lymphocytes. Extensive deposits of amyloid occur in the pyramidal portions of the organ. They have a definite pericapillary arrangement.

*Pararenal Ganglion:* A definite deposit of amyloid is present in the interstitial fibrous tissue of the ganglion.

No deposits of amyloid were found in the spleen, liver, adrenals, pancreas, prostate, brain or any organ or tissue other than those mentioned.

## DISCUSSION

It is not the purpose of this paper to explain the clinical, physiological, or biochemical principles involved in amyloidosis. Clinically, it is sufficient to say that our case deals with an individual 65 years of age who in the last three years of life developed a progressive left-sided heart failure which was twice recompensated before death — a cardiopathy with hypertension without renal disease leading to complete decompensation.

It is rather our purpose to confine the discussion to the anatomical observations. The review of these findings in our case at once demonstrates definite and important variations from the observations made in those cases previously reported by Steinhaus, Wild, and by Beneke and Bönning. But one thing is unconditionally common to the foregoing and the case under our observation, *i. e.* the

fibrillae concentrated in the peripheral portion of the cell. There is no evidence of degenerative change in these cells. It is of importance that in those instances in which this actual invasion of the cell has occurred, the pericellular tissues are often amyloid free except at that point at which the amyloid bud makes its invagination. It is further of importance that invaginating buds of amyloid are always traceable in serial sections to a point at which they become continuous with a pericapillary deposit of amyloid in the immediate vicinity.

There is no evidence of amyloid in the chordae tendineae, although it is present in abundance at the musculotendinous transition of the papillary muscles.

*Endocardium:* Focal collections of diffuse amyloid infiltration are here present as within the myocardium. The absence of large vessels in this tissue places the deposits, which here also are perivascular, about the capillaries and minute venules. Many of these deposits, because they appear primarily well myocardialward in this tissue are directly continuous or contiguous with the deposits of amyloid of the myocardium proper. In the right auricle alone the deposits have become sufficiently large, subendothelially to cause a protrusion of a large area of endothelium. The aortic valve is definitely infiltrated, especially in the proximal portion where the deposit of amyloid is continuous with that present in the myocardium. Peripherally in the valves the deposits consist of focal areas deep in the subendothelial tissues. Amyloid in similar distribution is found within the pulmonic, mitral and tricuspid valves. Never is it apparent from the surface. Its occurrence is always deep in the subendothelial stroma.

*Pulmonary Artery:* Distinct foci of amyloid are present in the outer media and adventitia of the pulmonary artery. In at least one instance vasa of the wall are demonstrable within the amyloid mass.

*Aorta:* Distinct foci of amyloid are apparent throughout the media. Many have a definite relationship to the vasa vasorum. A curious infiltration of the para-aortic adventitial veins is also evident. In this location there is a distinct localization of the amyloid within the media, though, at times the deposit has broken through and bulges the endothelium of the intima. There is no thrombus formation at the point of intimal protrusion.

*Inferior Vena Cava:* Distinct diffuse amyloid deposit is apparent,

which the amyloid deposits are slightly more extensive it is apparent that the amyloid has extended from its pericapillary nidus into the interstitial tissue which it obliterates, ultimately replacing the stroma of the muscle cells which it surrounds, bridging the entire interval from capillary wall to muscle cell; but always one observes that the lamellae which stratify these deposits have their centers in a capillary. In the replacement of interstitial tissues it is evident that those capillaries about which the amyloid is primarily deposited are compressed to minute strands and occluded, ultimately completely disappearing. It is of equal importance, that other capillaries remain dilated and congested in many amyloid areas, and while this condition maintains there is little evidence of cellular atrophy; but once this group of capillaries is gone, the amyloid masses are seen to be mottled by hollow lacunae, the rests of pre-existent muscle cells.

It is further observed that amyloid occurs often in the myocardial venules, never in the arteries; that the extremely dense areas of amyloid in which all tissue structure has been obliterated often correspond roughly to a single arteriovenous capillary tree. In the endocardium and valves, amyloid deposit occurs only in the deeper tissues. Likewise in the aorta and pulmonary arteries the amyloid occurs primarily in the media, the site of the vasa vasorum; it presents in the intima only by continuity from medial deposits; and finally, it has been noted that in the papillary muscles of the ventricles, while amyloid is present abundantly in the muscle it is entirely wanting in the chordae tendineae, abruptly ending at the musculo-tendinous transition line. It is therefore apparent that amyloid deposit does not occur in those tissues which are avascular except by extension from deposits in vascular tissue; that it occurs in vascular tissue primarily in relation to venous capillary endothelium and vein walls, never in arteries except the pulmonic artery and aorta where it is present about the medial vessels and adventitial veins. Moreover the distribution and abundance of the deposits bear a specific relation to the area of the capillary bed. It is my opinion that the amyloid is deposited only in those tissues which are known to have a definite vascular bed, and here its deposition is proportional to the vascular bed; and its deposit is primary about the endothelium of specific venocapillaries and venules from which it expands to include adjacent structures. Ultimately it cuts off the

primary localization of amyloid in the heart, especially the myocardium.

At autopsy, the heart, an organ of 700 gm., is dilated and hypertrophic throughout, and possessed of peculiar properties suggestive of amyloid deposition. In this lies the first variation of our case from all others in that the heart is markedly hypertrophic, whereas in all previously reported cases it was found to be atrophic. Unquestioned deposits of amyloid are not grossly recognized in the heart at autopsy, a feature contrary to the case of Beneke and Bönnig in which the amyloid was readily detectable as large glassy nodules within the heart wall. Recognized tests for amyloid, however, reveal in our case a diffuse amyloid infiltration of the heart. In the premise the diffuse type of amyloid infiltration of the heart in this case bridges a gap in what has been previously known of primary myocardial amyloidosis, in that it demonstrates the possibility of diffuse as well as localized types of amyloid deposit in primary myocardial amyloid infiltration; a feature which places the primary myocardial amyloidosis in the same category as the generalized types of amyloidosis, in which both focal and diffuse types of amyloid deposit have been repeatedly observed, for example, in the spleen.

The microscopic findings place the only detectable amyloid deposits in this case in the heart and the neighboring great vessels, the lungs, the kidney, and a perirenal ganglion. In the heart the amyloid is deposited diffusely, though irregularly, throughout the tissues. In the epicardium it is present only in relation to veins some of which it occludes by pressure from without, and which form the centers from which it radiates into the adjacent tissue replacing all but the fat cells. In the endocardium it is present only in the deeper layers of the stroma and here again it is in definite relation to veins. In the less densely infiltrated areas of the myocardium, it is present primarily immediately adjacent to specific capillary endothelium whose connective tissue stroma it replaces; while other capillaries are entirely free of this deposit. This constitutes pre-eminently the primary site of amyloid deposit. Its occurrence in isolated areas in the interstitial tissue or about muscle cells is in every instance traceable in serial sections to a pericapillary relation. In the presence of slight traces of amyloid, therefore, it is located primarily about capillary endothelium. By examination of fields in



fields in which a pericapillary amyloid bud simply contracts with one side of a muscle cell as well as in fields in which the cell is at certain levels densely surrounded. There is evident from serial cross-sections an actual invasion of the cell by amyloid, with penetration of the cell wall by the amyloid which within the cell extends bipolarly leading to peripheral concentration of the myofibrillae and lateral nuclear displacement. This intracellular amyloid lies in the position of the sarcoplasm. The entire group of intracellular structures in cells invaded in this manner are intact and manifest no changes other than displacement. There is no evidence of vacuolization or other cell response usually seen in the presence of intracellular foreign bodies. This observation presents a feature thus far entirely foreign to our knowledge of cellular pathology. The actual penetration of a fixed cell wall by a foreign substance without cellular destruction or recognizable alteration other than displacement is unique. The fraying out of the cells at the portal of entry of these invading buds offers many possibilities as to the nature of the muscle cell wall, which hitherto have been unsuspected.

The serial cross-sections, therefore, establish the fact that the apparent indiscriminate invasion of the muscle cells from the peripheral concentration zones is purely a sectional and distortional artifact. Cellular invasion occurs only by the method which we have just described, and by that method in comparatively few instances.

The deposits in the lungs and kidneys are likewise pericapillary in distribution. We therefore find no substantiation for the opinion advanced by Beneke and Bönning to the effect that amyloid is deposited in the ectoplasmic zone of the tissue cells as a result of disturbed metabolic equilibrium, which ultimately by encasement of the cell by amyloid leads to inanition atrophy. In fact we find no evidence that the amyloid bears relation to disturbed metabolism in any specific cell. It is our opinion that amyloid is deposited from the tissue lymph as a result of changes in the permeability in the venous endothelium. Its presence may be simply the result of abnormalities in the capillaries which are non-receptive to a substance to which normally they may be permeable.

arterial supply to the part and this in turn leads to atrophy of the structures in the involved area with a persistence of amyloid. Amyloid apparently is not affected by the anemia but continues to accrue, leading ultimately to closure of the lacunae left by atrophic muscle cells. A sheet of amyloid is thus produced which is marked by whorls, whorls formed by concentration of contingent amyloid deposits which encroach from several directions on muscle cells and lead to concentration bands of amyloid about individual cells, prior to the time the vascular supply has been severed.

It has not, however, in any instance been my observation that amyloid occurs primarily in the ectoplasmic zone of the muscle cells from which it invades the cell at random as suggested by Beneke and Bönning. Indeed, as has already been pointed out, the primary deposits of amyloid occur about venocapillary endothelium, from which it extends to surround muscle cells which in densely infiltrated areas it encases. In these areas in which the muscle cells are entirely surrounded, many of the cells manifest no evidence of atrophy. Rather the myofibrillae and other intracellular components are simply concentrated, as by partial dehydration. In serial section these cells have been traced in every instance to a level at which they reach an amyloid-free field. Actual cell atrophy is apparent only when in serial cross-sections the amyloid is so abundant as to have led to complete vascular occlusion to the area and to the cell over its entire extent. Of those cells which show no evidence of atrophy, many are indented and invaginated by adjacent amyloid buds; more commonly they are symmetrically compressed by amyloid which forms a pressure concentration zone at their periphery. From these encasements many cells, their membranes intact, have retracted in fixation. There is microscopically no evidence that amyloid invades the cell from this periphery except by one method which will later be described. It is apparent that by far the greater amount of amyloid replacement occurs as follows: the cell first dies by loss of its blood supply, leaves its empty lacuna in a field of amyloid and, then only, amyloid expands to fill this interval. This feature explains the picture of multiple whorls so often seen in a field of solid amyloid.

Microscopically evidence is found that amyloid actually invades the cell by only one method, a method independent of the concentration of the pericellular amyloid, inasmuch as it occurs in

## DESCRIPTION OF PLATES

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### PLATE 32

FIG. 1. Gross photograph of heart.

FIG. 2. Right auricle. Iodin-sulphuric acid test. Reflected light.  $\times 13$ .

## SUMMARY

1. The distribution of amyloid within the myocardium in primary myocardial amyloidosis may be diffuse as well as focal.
2. Its deposition within the heart may occur in the presence of hypertrophy as well as atrophy.
3. It is deposited only in those tissues which have a known vascular bed. Its presence in avascular tissue is only accomplished by continuity with deposits in vascular tissue.
4. The deposition of amyloid occurs primarily about venocapillary endothelium from which it extends to surround the normal tissues, ultimately cutting off the vascular supply to the part. Then only the tissues atrophy and are replaced by amyloid. This constitutes the primary mode of amyloid infiltration.
5. Amyloid gains entrance to occasional cardiac muscle cells by a process of invagination and ultimate penetration of the cell wall. This is a direct method by which myocardial cells may be replaced by amyloid.
6. There is no evidence that amyloid deposit is dependent on localized metabolic changes, nor is there evidence of primary pericellular deposition of amyloid, from which it freely invades living cell substance.
7. The deposit of amyloid apparently is dependent upon changes in endothelium, especially of venocapillaries, which may possibly become impermeable to some substance in the tissue lymph which may normally be present in tissue lymph and capable of permeating venocapillary endothelium.

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PLATE 33

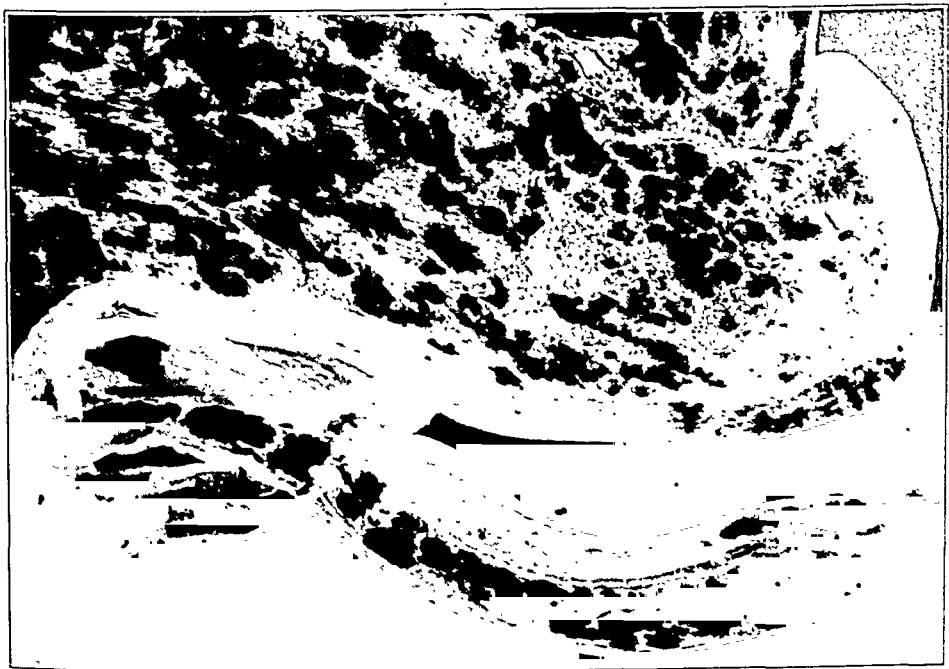
FIG. 3. Left ventricular myocardium. Iodin-sulphuric acid test.  $\times 13$ .

FIG. 4. Lung. Pericapillary amyloid in alveolar wall.  $\times 114$ .

FIG. 5. Left ventricular myocardium. Diffuse amyloid deposit.  $\times 540$ .



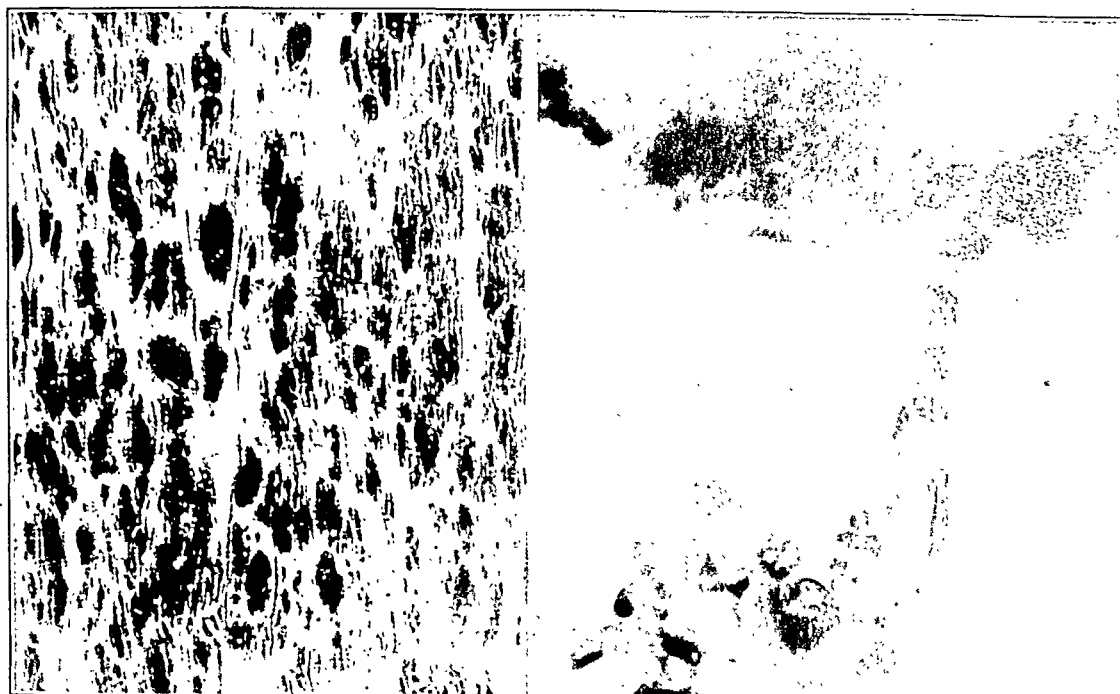
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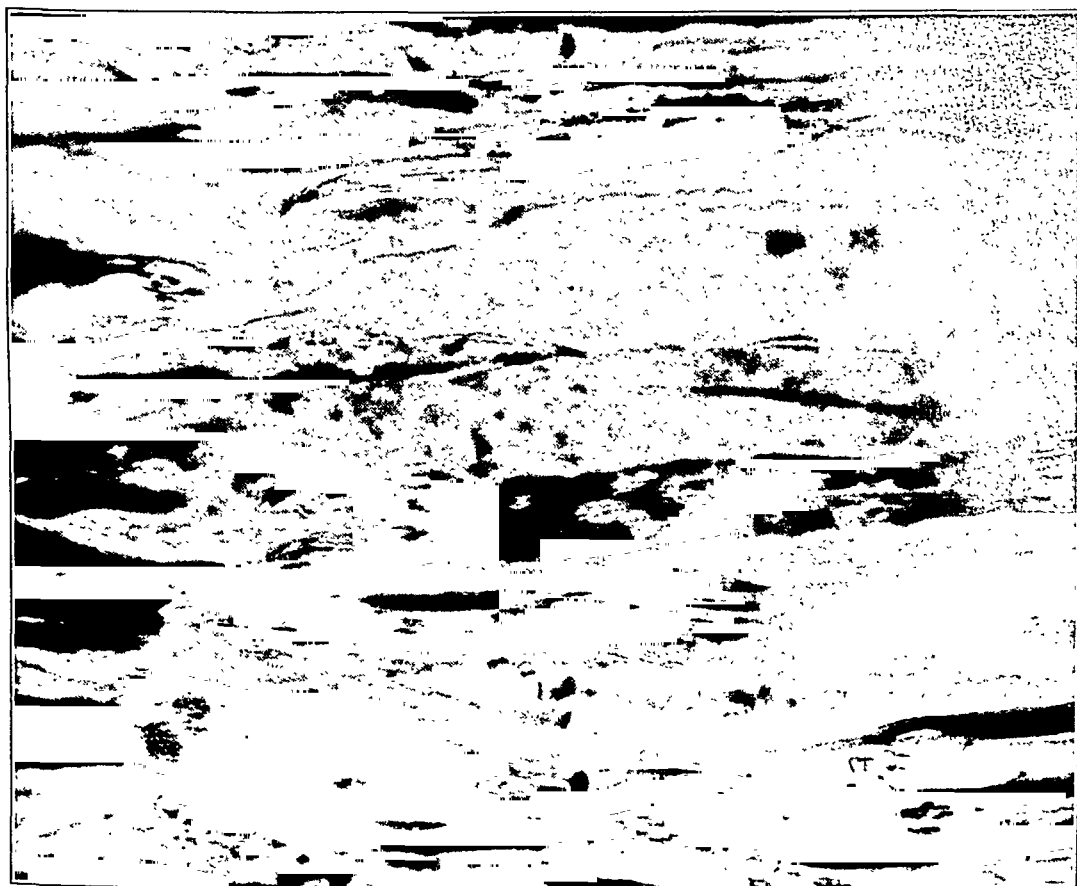
PLATE 34

- FIG. 6. Myocardium. Pericapillary deposits of amyloid with partial capillary occlusion in left field.  $\times 1000$ .
- FIG. 7. Myocardium. Abrupt termination of muscle fibers at a point at which capillaries pass deep into tissue.
- FIG. 8. Myocardial cell invaginated by amyloid.  $\times 2000$ .
- FIG. 9. Same cell as Fig. 8, in serial section showing penetration of amyloid into sarcoplasm. The mass of amyloid is continuous with that in Fig. 8.  $\times 2000$ .



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4



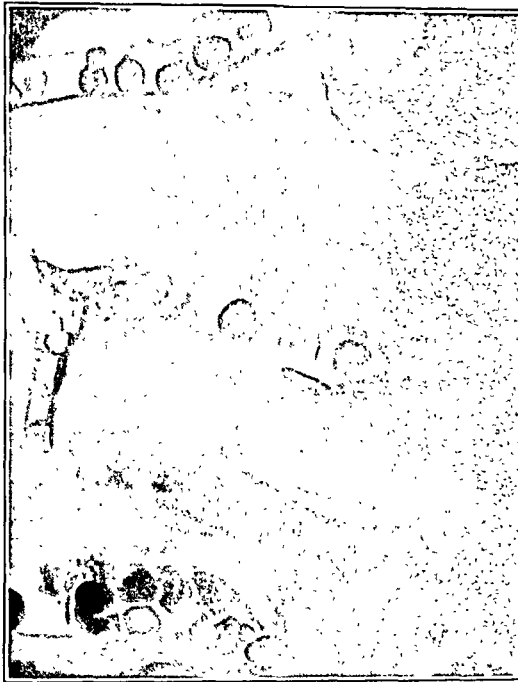
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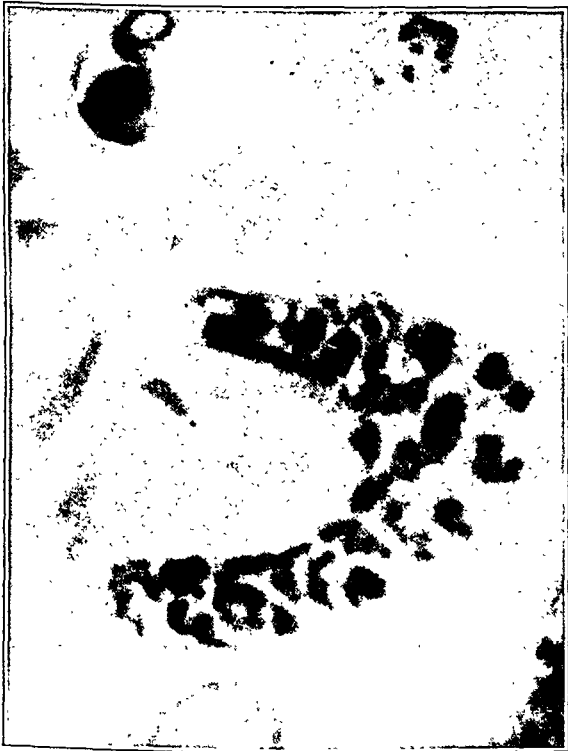




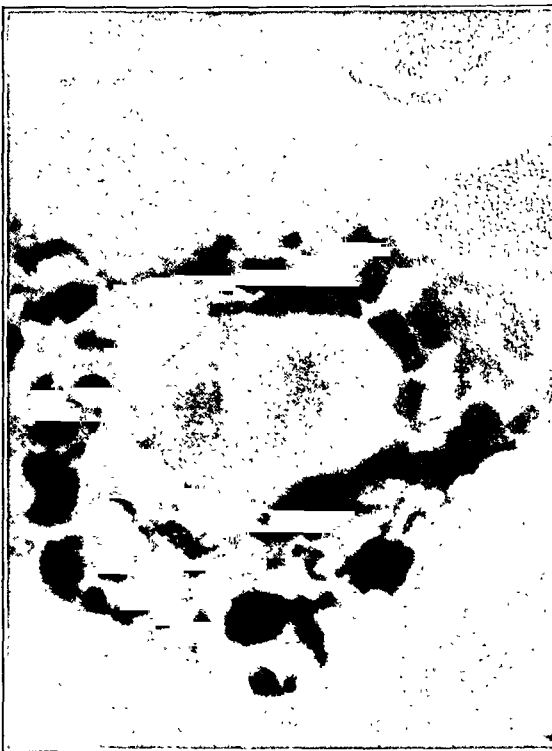
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9

Larsen

Primary Myocardial Amyloidosis

lesions which might well have accounted for the production of amyloid.

Three cases of local amyloidosis of the seminal vesicles are reported by Erlach.<sup>6</sup>

Mallory<sup>7</sup> has seen one case of amyloidosis of the bladder.

### CASE REPORT

*Clinical Note:* A. J. No. 4557. Female, 54 years of age. Referred to Collis P. Huntington Memorial Hospital Oct. 5, 1928, by Dr. R. B. Greenough for study of tongue. For past fourteen months she had had "canker sores" and for past six months swelling of left and right submaxillary regions. Tongue showed smooth patches of epithelium with indurated foci beneath on both sides, and on the right side a deep ulcerated focus. Biopsy was done by Dr. C. C. Simmons and a pathological diagnosis of leukoplakia with a question of beginning carcinoma was made. It was noted that the underlying fibrous tissue was extremely dense.

General physical examination was essentially negative except that there was no excursion of the diaphragm, the abdominal muscles were tense and the walls of the blood vessels were palpable. The Wassermann test was negative. Examination of the blood showed only slight secondary anemia. Nov. 27, 1928, a general increase in size of the tongue was noted and a diffuse painless thickening appeared within both cheeks. Several low voltage X-ray treatments were given the tongue without producing much change. March 7, 1929, examination by Dr. J. C. Aub showed the tongue markedly enlarged, thickened and hard, the muscles of the shoulder girdles hard and tense, whereas the muscles of the upper arm were soft and flabby. The muscles of the thighs and abdomen were hard and tense. April 9, 1929, patient died at her home without definite clinical findings, and an autopsy was performed at the Palmer Memorial Hospital.

### AUTOPSY FINDINGS

*Body:* Is that of a well developed and fairly well nourished elderly white female. Weight about 135 pounds. Rigor mortis slight. No postmortem lividity. Musculature of chest wall and abdomen extremely tense. Musculature of shoulders prominent. Both thighs considerably enlarged, left more than right, musculature very hard and firm. Slight edema of both legs. Musculature above both shoulders firm and hypertrophied.

*Head:* Moderate amount of gray hair. Contour of skull normal. Pupils equal, regular, 4 mm. in diameter. Ears and nose negative. Firm, symmetrical swellings at both angles of mouth. Tongue firm, rubbery and protrudes slightly from mouth, bulging between teeth laterally.

## GENERALIZED AMYLOIDOSIS OF THE MUSCULAR SYSTEMS\*

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Amyloidosis of parenchymatous organs, particularly of the liver, spleen and kidneys, is too commonplace to attract attention. However, amyloidosis of other structures is rare. The so-called amyloid tumors, while rare, are fairly well known. The tongue<sup>1</sup> and upper respiratory passages are the usual sites.<sup>2</sup>

Amyloidosis involving the heart has been reported. Kann<sup>3</sup> describes a case in a 77 year-old male dying of carcinoma of the esophagus, complicated by tabes dorsalis, and with a history of chronic alcoholism. The heart was markedly hypertrophied, the left ventricle measuring 1.9 cm. in thickness. There was diffuse amyloid surrounding the myocardial fibers, causing more or less atrophy of them. The blood vessels of the heart as well as the pulmonary artery with its vasa vasorum showed marked amyloidosis, particularly in the media. The bronchial lymph nodes showed tuberculosis.

Steinhaus<sup>4</sup> describes a case in a 40 year-old male with profuse rectal hemorrhage as the chief clinical feature. At autopsy generalized amyloidosis of the heart, stomach and intestine was found, the musculature appearing in gross homogeneous and glassy. No underlying process which could explain the condition was detected.

In three cases reported by Lubarsch,<sup>5</sup> widespread amyloidosis of the muscular systems occurred. In the first case, a man 54 years of age, empyema was found. The heart, skeletal and smooth muscle, and the corium all showed amyloidosis. In his second case, a woman 66 years of age, who died of purpura hemorrhagica and mitral endocarditis, amyloid was deposited in the heart, tongue, skin and smooth muscle. Carcinoma of the pylorus was the cause of death in the third case, a 45 year-old man, whose heart, intestinal musculature, spleen, lymph nodes and prostate were the sites of amyloid deposition. This group, with the possible exception of the first, had

\* Received for publication November 7, 1929.

above a point of partial obstruction. However, no obstruction made out. Otherwise negative. Marked muscular thickening about anus.

*Liver:* Weight 1230 gm. Capsule smooth. On section markings distinct, purplish red in color.

*Gall Bladder:* Wall 0.4 cm. thick, firm and gray. Contains approximately 80 cc. of yellowish bile. Bile ducts patent.

*Adrenals:* Negative.

*Kidneys:* Right 145 gm., left 155 gm. Reddish brown in color. On section normal markings distinct. Capsule strips readily from smooth surface. Glomeruli visible. Pyramids well marked. Pelves negative. Ureters negative.

*Bladder:* Wall considerably thickened, elastic and pale.

*Genitalia:* Two large, pedunculated, subserous, calcified, irregular masses, one 6 cm., the other 8 cm. in diameter, are adherent to fundus of uterus. On section uterus negative. Tubes and ovaries negative. External genitalia negative.

*Veins:* Wall of vena cava half again as thick as normal and somewhat stiff.

*Aorta:* Scattered, deep yellow atheromatous plaques in abdominal and thoracic portion. At left of celiac axis aorta compressed and forced to left by hypertrophied crura of diaphragm.

*Thyroid:* Negative.

#### MICROSCOPIC EXAMINATION

*Heart:* Between muscle fibers and in many instances compressing them is a hyaline acidophilic deposit fairly abundant and in some places causing practically complete atrophy of fibers. Endocardium somewhat thickened and irregular with similar glossy, translucent material.

*Lung:* Moderately congested. Alveoli contain large numbers of phagocytes distended with fat droplets, sometimes attaining a very considerable size. At one point these are surrounded by fibrous tissue. Media of blood vessels somewhat thickened and acidophilic with scattered compressed cells present. Pleura moderately thickened by fibrous tissue and small amounts of acidophilic hyaline material.

*Diaphragm:* Serosal surface greatly thickened by fibrous tissue

*Primary Incision:* Y type. Panniculus adiposus 4 mm. thick. Fat translucent and watery. Musculature of abdominal wall grayish and tough, cutting like gristle.

*Peritoneal Cavity:* Surfaces smooth and shining. No free fluid. Appendix thickened, 8 cm. long, retrocecal. Diaphragm 4th rib right, 5th space left. Mesenteric lymph nodes slightly enlarged. Cisterna chyli considerably dilated.

*Pleural Cavities:* Right, surfaces smooth, contains approximately 100 cc. of clear, straw-colored fluid. Left almost completely obliterated by dense fibrous adhesions.

*Pericardial Cavity:* Surfaces smooth and shining but pericardium definitely thickened and stiff.

*Heart:* Weight 410 gm. Over epicardium along vessels are rough, thickened, gray foci. Wall of left auricle is strikingly thickened and leathery, measuring 0.7 cm. in diameter. On section no muscle fibers seen in auricular walls, which are white and firm. On lateral aspect of right ventricle just below tricuspid valve is a firm white mass embedded in musculature measuring 1.5 by 2.5 cm. in diameter. Near apex of left ventricle is a firm whitish mass fading imperceptibly into musculature measuring 3 by 2.5 by 1 cm. This is covered by epicardium. On section these foci are white, glistening, tough and show traces of preëxisting markings. No abnormalities of valves noted. Endocardium negative. Coronaries thickened but not calcified.

*Lungs:* Both lower lobes dark red, injected and meaty. From left lower lobe a moderate amount of grayish red thick fluid can be expressed. Anterior mediastinum practically obliterated by thickened fibrous tissue which overlies pericardium and causes it to adhere closely to under surface of sternum.

*Diaphragm:* Cuts with marked resistance. Both serosal surfaces white and thickened and musculature is dry, gray and gristly. Diaphragm ranges from 0.5 to 1 cm. in thickness. Both crura of diaphragm are greatly thickened, right more than left, and they compress aorta.

*Spleen:* Weight 110 gm. Capsule smooth. On section normal markings distinct. Slight amount of pulp is scraped away.

*Pancreas:* Weight 85 gm. Negative.

*Gastro-Intestinal Tract:* Entire musculature moderately thickened, tough and elastic, suggesting hypertrophy of musculature

*Breast:* Marked irregularity of structure. Some epithelial hyperplasia in ducts. Much connective tissue is hyalinized and gives reactions typical of amyloid. At one point there is a considerable portion of osteoid tissue and bone with a formation of somewhat fibrotic marrow.

*Lymph Node:* Moderate hyperplasia.

*Aorta:* Moderate thickening of intima. Some fibrosis of media.

*Mesenteric Vessels:* Marked infiltration of media by hyalinized material giving characteristic staining reactions of amyloid.

*Striated Muscle (Rectus Abdominis):* Broad strands of hyaline material with occasional compressed fibroblasts. Also, compressed muscle cells set in a matrix of hyaline acidophilic material giving typical staining reactions of amyloid.

*Smooth Muscle:* Atrophic fibers embedded in homogeneous matrix with typical staining reactions of amyloid.

*Cheek:* Beneath corium, masses of hyaline material compressing fibroblasts and surrounding and compressing striated muscle fibers. This material gives typical staining reactions of amyloid.

*Tongue:* Connective tissue contains large masses of hyaline material giving typical staining reactions of amyloid. Similar substance in media of blood vessels and surrounding and compressing striated muscle fibers. Moderate endothelial and lymphocytic infiltration beneath epithelium.

*Anatomical Diagnoses:* Amyloidosis of heart, diaphragm, intestine, stomach, esophagus, muscles of thighs, chest, abdomen, tongue and cheeks, bladder, gall bladder, arteries and veins; bronchopneumonia; healed pleuritis, left; hydrothorax, right; leiomyoma of uterus with amyloidosis.

## DISCUSSION

Amyloidosis is practically always associated either with wasting diseases, chronic inflammation or a tumor process. In the present case, as in the case of Steinhaus, we seem to be dealing rather with a widespread perversion of function of the connective tissue elements of the muscular structures of the body, involving smooth muscle, striated muscle and heart muscle. There is no indication of any process which might have brought about this change in function. The extremely unusual finding of bone in the stroma of breast tissue

with slight lymphocytic infiltration. Only few nuclei in fibrous tissue, and pale acidophilic hyaline material giving the typical reactions for amyloid present. Media of blood vessels considerably thickened and hyalinized. Muscle fibers in diaphragm atrophic and some have entirely disappeared and are embedded in a very dense, almost acellular, hyaline acidophilic matrix, giving the typical reactions for amyloid.

*Spleen:* Hyaline thickening of intima of smaller blood vessels and of media of larger blood vessels. Otherwise negative.

*Pancreas:* Slight postmortem change. Otherwise negative.

*Stomach:* In outer portion of muscularis muscle cells compressed and in many places replaced by hyaline, amorphous, acidophilic substance giving the typical reactions for amyloid.

*Small Intestine:* Similar material involving both circular and longitudinal muscular coat. In some places this change is so marked in the longitudinal coat that only occasional atrophic fibers are present in a wide expanse of hyaline matrix. Mucosa essentially negative.

*Liver:* Some hepatic arterioles almost obliterated by marked hyaline acidophilic infiltration of media. Liver cells essentially negative. Some slight congestion. Sinusoidal endothelium slightly more prominent than usual.

*Gall Bladder:* Wall markedly edematous. Blood vessels show hyaline material in media.

*Adrenal:* Slight increase in fibrous tissue involving zona glomerulosa. Hyaline infiltration of media of blood vessels.

*Kidney:* Congestion. Some sclerosed glomeruli. Edema in some tubules. Larger vessels show hyaline infiltration of media.

*Bladder:* Scattered atrophic small muscle fibers embedded in a very dense acidophilic, homogeneous hyaline stroma giving typical reactions of amyloid. Large deposits of similar substances in walls of arteries and veins.

*Uterus:* Small, partly encapsulated nodule shows peripherally interlacing bands of fibers embedded in a somewhat hypertrophied stroma and showing centrally only occasional scattered muscle fibers embedded in a dense hypertrophied stroma giving typical reactions of amyloid. Remainder of uterine musculature in some portions normal, in other portions atrophic and embedded in homogeneous matrix giving reactions of amyloid.



## DESCRIPTION OF PLATES

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### PLATE 35

FIG. 1. Median section through heart showing replacement of wall of left auricle by amyloid, mass of amyloid near apex, and small foci of amyloid about blood vessels in ventricular walls. A small portion of right auricle with walls similar to left is shown.

FIG. 2. Wall of left ventricle showing extensive amyloid deposits and extreme hydropic degeneration of myocardial fibers.       $\times 200$ .      1

showing only slight chronic mastitis suggests that this amyloidosis is perhaps the expression of a widespread dysfunction of fibroblasts.

In the light of the distribution of the substance, there can be no question but that it is the product of abnormal fibroblastic activity as in many places it has formed at a considerable distance from blood vessels. A striking feature is the relatively large amount of amyloid laid down in some places in the heart, forming in gross discrete tumor masses, and in the longitudinal muscular coat of the small intestine, the muscle fibers being separated from one another by interspersed deposits of amyloid more than five times the diameter of the fibers themselves.

There is no evidence of amyloidosis in the organs usually affected, such as the liver, spleen and kidney, other than a slight involvement of the media of their blood vessels.

### SUMMARY

A case of generalized amyloidosis of the muscular systems is reported. The evidence indicates a widespread perversion of fibroblastic function. The parenchymatous organs are not involved.

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PLATE 36

FIG. 3. Diaphragm. Marked deposit of amyloid with atrophy of muscle fibers.  
× 200.

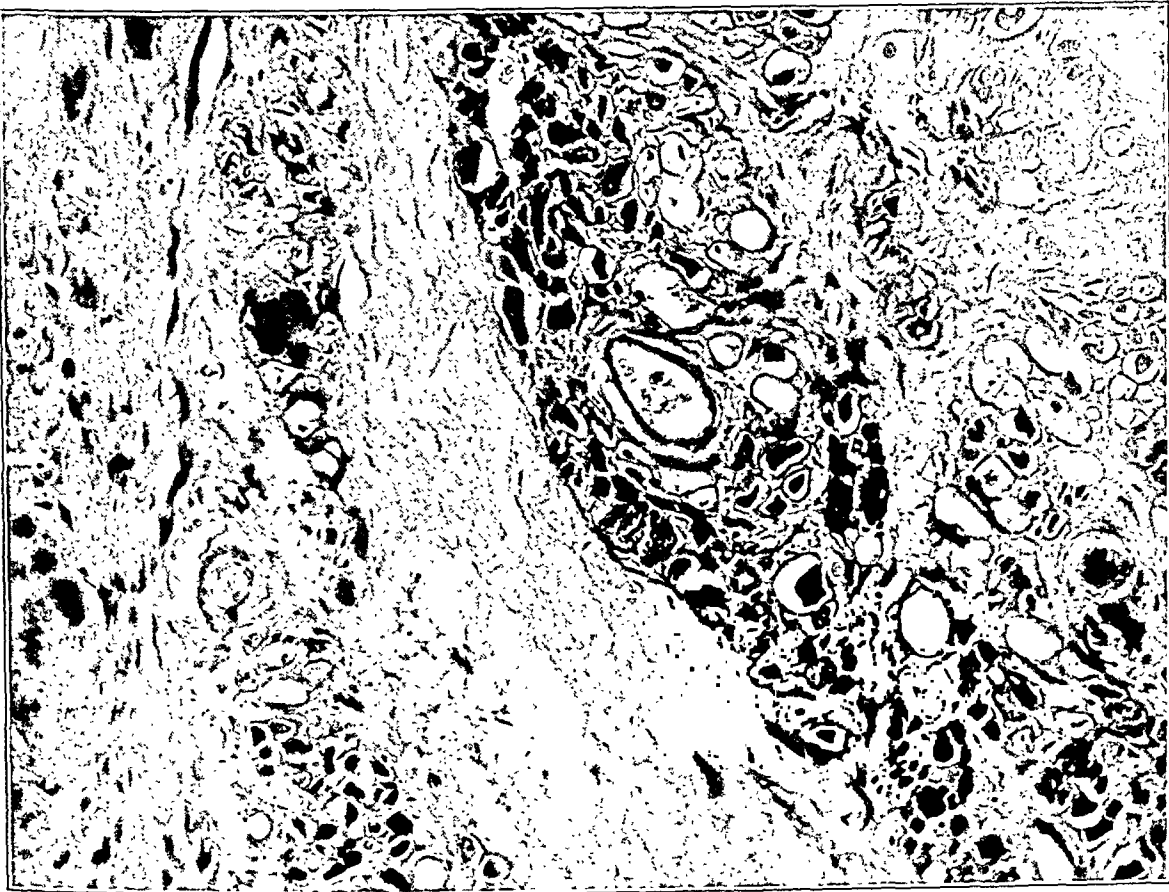
FIG. 4. Deposition of amyloid in muscularis of stomach showing marked atrophy of smooth muscle fibers.      × 200.

FIG. 5. Tongue showing replacement of striated muscle by hyaline connective tissue and amyloid.      × 200.

FIG. 6. Formation of bone in stroma of breast.      × 200.



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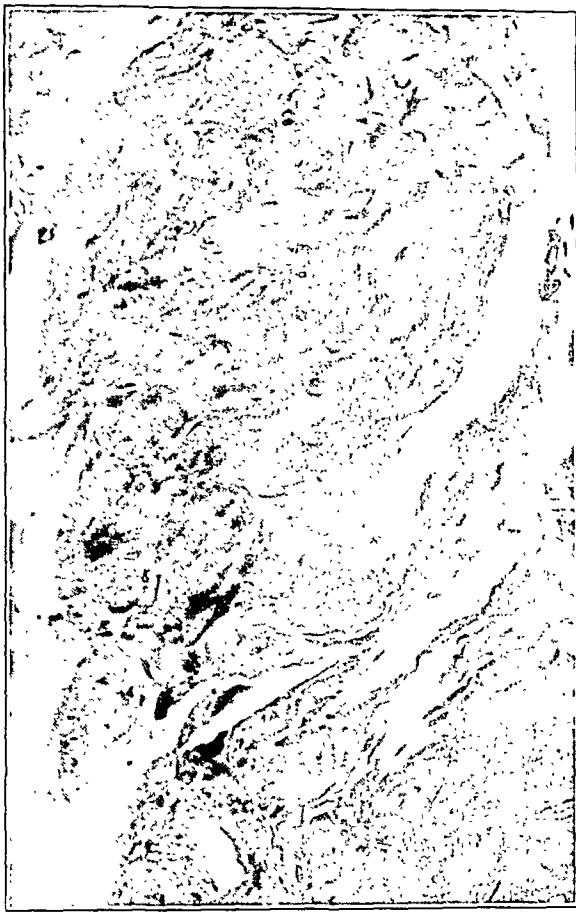


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being much greater however in the lesions where fowl-pox was a factor. This same difference in the extent of the swelling was noted after seven days. After three days, five days and seven days pieces of skin from each of the three types of lesions were removed for microscopic sections.

Since the Guarnieri bodies of vaccinia are reported as being more readily demonstrated in the cornea than in the skin of the fowl,<sup>4</sup> corneal inoculations with the vaccinia virus were made on seventeen chickens as well as on a large number of rabbits and rats. Some of the chickens were immune to fowl-pox while others were normal. The condition of immunity to fowl-pox apparently made no difference in the chicken's response to the vaccinia virus.

For comparison with the vaccinia lesions a number of chickens were inoculated on the cornea with fowl-pox virus. All of the fowl-pox inoculations were made with the virus which we have used experimentally for the past two and a half years.<sup>5</sup> For the various vaccinia inoculations four different samples of stock vaccine (Squibb) were used, as well as a substrain of the Levaditi neurovaccine.

### MICROSCOPIC FINDINGS

The marked contrast in the histology of the vaccinia and fowl-pox lesions is illustrated in Figs. 1 and 2. Each figure represents a section from a seven-day lesion. In the vaccinia lesion, Fig. 1, there is very little epithelial hypertrophy and hyperplasia compared to the great epithelial overgrowth in Fig. 2. In the latter section the swollen feather follicles make tumor-like projections into the subcutaneous tissue, or appear as circular masses of cells where they have been cut transversely. The inflammatory reaction, on the other hand, is more pronounced in the vaccinia lesion which shows the subcutaneous tissue heavily infiltrated with lymphocytes and large mononuclear cells. Both sections show a considerable amount of fibroblast proliferation as well as a definite infiltration with eosinophiles. Guarnieri bodies were not found in the skin of the chick, although chicken corneas inoculated with the same vaccinia virus showed typical Guarnieri bodies.

In the mixed lesion, due to the two viruses, the fowl-pox predominates, presenting a picture similar to that shown in Fig. 2. Except

# A COMPARISON OF THE LESIONS OF FOWL-POX AND VACCINIA IN THE CHICK WITH ESPECIAL REFERENCE TO THE VIRUS BODIES\*

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The study of filtrable virus diseases has repeatedly been complicated by observers who have claimed an identity of unrelated viruses. The attempt to prove that the virus of fowl-pox will produce the characteristic lesions of vaccinia in rabbits and in man was refuted by Andervont<sup>1</sup> in a careful study of the subject. Recently work has appeared in which the characteristic lesion of fowl-pox in the chicken is reported to have been produced by the vaccinia virus. Ludford<sup>2</sup> claims that "the virus bodies produced by vaccinia in epidermal cells of the skin and cornea of the chick are exactly the same as those of fowl-pox." My own experience with the virus bodies in the two diseases in question has been so completely different that a series of experiments was started in the hope of clarifying the matter.

## EXPERIMENTAL WORK

Six small chicks (4 weeks) were prepared for inoculation by plucking the feathers from the sides of the head (technique suggested by Findlay<sup>3</sup>). Two of the chicks were inoculated with stock vaccinia virus (Squibb), two with fowl-pox virus alone, and two with fowl-pox virus mixed with vaccinia virus. The chicks were kept in separate cages to prevent accidental infection.

Three days after the inoculation, the skin on the heads of all the chicks was found to be slightly indurated, and the feather follicles swollen. The lesions caused by the fowl-pox virus alone were pale, while the other four lesions appeared slightly inflamed. After five days the swelling had become more pronounced on all of the chicks,

\* Work done while a Fellow in Medicine of the National Research Council.  
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Ludford,<sup>2</sup> in his description of the virus bodies produced in chick epithelium by the vaccinia virus, mentions the lipoidal coating of the bodies. Moreover he concludes his paragraph on this lesion with the sentence: "This study of the vaccinia virus inclusions in cells of the epidermis of the chick has shown . . . that both as regards their structure and their mode of origin, they are identical with the virus bodies of fowl-pox . . ."

Findlay,<sup>3</sup> writing in the same volume, finds no serological relationship between the viruses of vaccinia and fowl-pox, yet he is apparently ready to accept without question the statement that "the virus of vaccinia produces exactly similar lesions in the skin of the chick to those caused by the virus of fowl-pox . . ." Ludford, in his communication, gives credit to Findlay for his pathological material, so it is questionable who is responsible for the error we feel sure has occurred. In any case we can see no other explanation for Ludford's work than that he was either mistakenly dealing with a fowl-pox lesion or with a lesion due to the mixed viruses of vaccinia and fowl-pox in which the inclusions of the latter predominated.

### SUMMARY

1. The histology of lesions obtained in chick epithelium following inoculation with vaccinia virus alone, fowl-pox alone, and the two viruses mixed, is described.

2. The characteristic virus bodies of fowl-pox, whether occurring in the skin or the cornea of the chick, give a positive reaction for fat. The Guarnieri bodies of vaccinia do not stain for fat.

3. Ludford's statement regarding the identity of the virus bodies of vaccinia and fowl-pox in the chick is refuted. His observations are due, it is believed, to the utilization of tissue from a mixed lesion of fowl-pox and vaccinia.

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for the more marked infiltration of lymphocytes and large mononuclear cells in the mixed lesion this could not be differentiated from the lesion due to fowl-pox alone.

The most characteristic difference between the lesions produced by the two viruses is seen in the virus bodies. In the skin of the chick no virus bodies whatsoever were found following inoculation with vaccinia virus, while, in the cornea, the largest Guarneri bodies of vaccinia were rarely found one-fifth the size of the Bollinger bodies of fowl-pox. Figs. 3 and 4 indicate the difference, the black bodies in Fig. 3 representing Bollinger bodies magnified 300 diameters, while the three central cells at the bottom of Fig. 4 contain Guarneri bodies magnified 1200 diameters. Moreover we have found that in the cornea of the chick, as in that of the rabbit and rat, Guarneri bodies may best be demonstrated forty-eight to seventy-two hours after inoculation, while the virus bodies of fowl-pox reach their maximum size only after seven to ten days.

Aside from these differences in size and time of appearance the virus bodies of fowl-pox, whether occurring in the skin or the cornea of the chick, are invariably found to have a lipoid component as demonstrated by fat stains of frozen sections, Fig. 3. In Guarneri bodies, on the other hand, we have never obtained a positive fat reaction, and numerous sections have been stained for fat from both skin and corneal lesions in chickens, rabbits and rats. This difference in their reaction to fat stains we have found to be an infallible criterion in differentiating between the virus bodies of fowl-pox and vaccinia.

## DISCUSSION

Our experiments confirm the observations of Andervont<sup>1</sup> that characteristic Guarneri bodies result from the inoculation of vaccinia virus on the cornea of chickens. These bodies, due to their size, time of appearance, and their failure to be colored by fat stains, cannot possibly be confused with the virus bodies of fowl-pox. However, the inoculation of mixed vaccinia and fowl-pox viruses on the skin of the chick produces a lesion in which the picture of fowl-pox predominates, with the formation of virus bodies characteristic of the latter disease.

## DESCRIPTION OF PLATES

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### PLATE 37

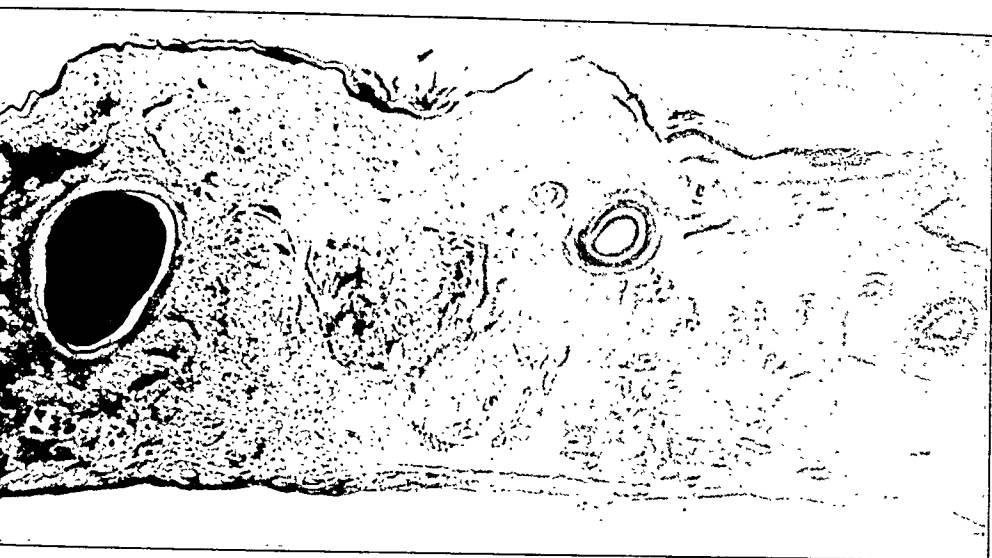
FIG. 1. Vaccinia lesion in skin of chick, seven days after inoculation. There is a marked infiltration of the subcutaneous tissue with lymphocytes and large mononuclear cells. The feather follicle at the left is filled with necrotic cellular débris. Hematoxylin and eosin.  $\times 40$ .

FIG. 2. Fowl-pox lesion in skin of chick, seven days after inoculation. The extreme swelling of all the epithelial structures is apparent. The dark bodies in the swollen epithelial cells are the virus bodies. Hematoxylin and eosin.  $\times 40$ .

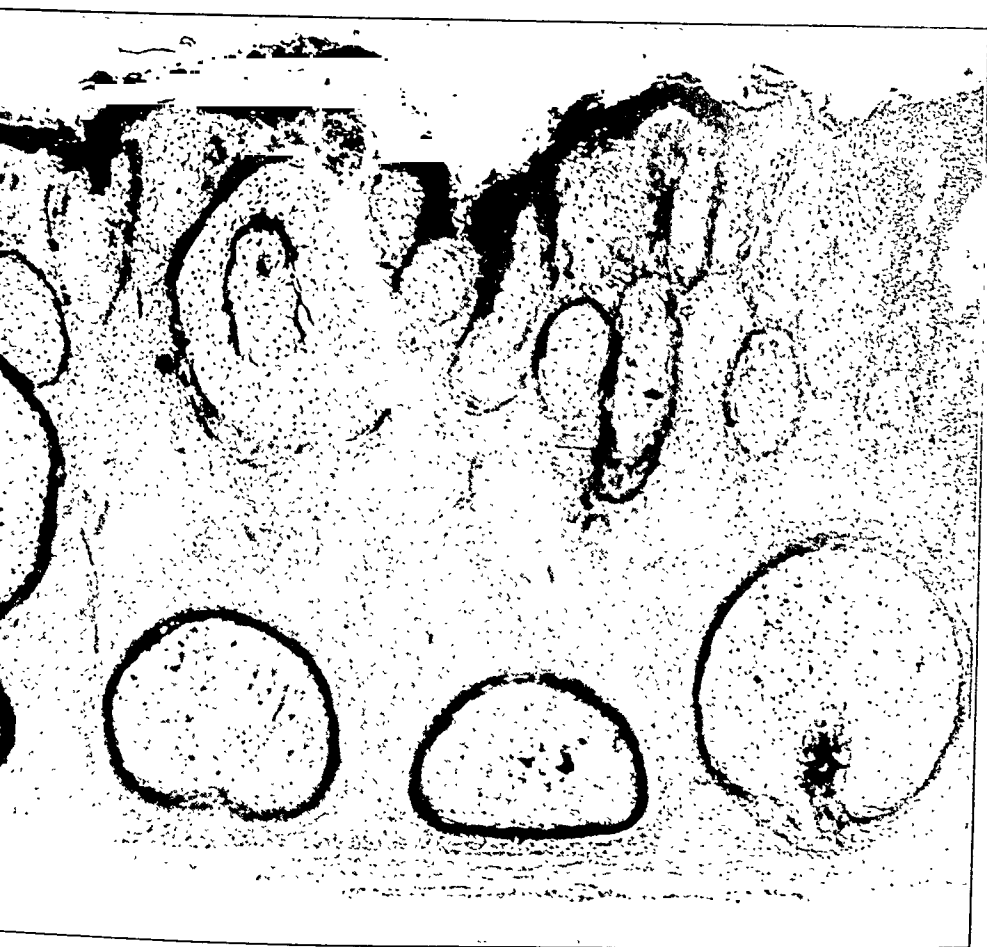
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PLATE 38

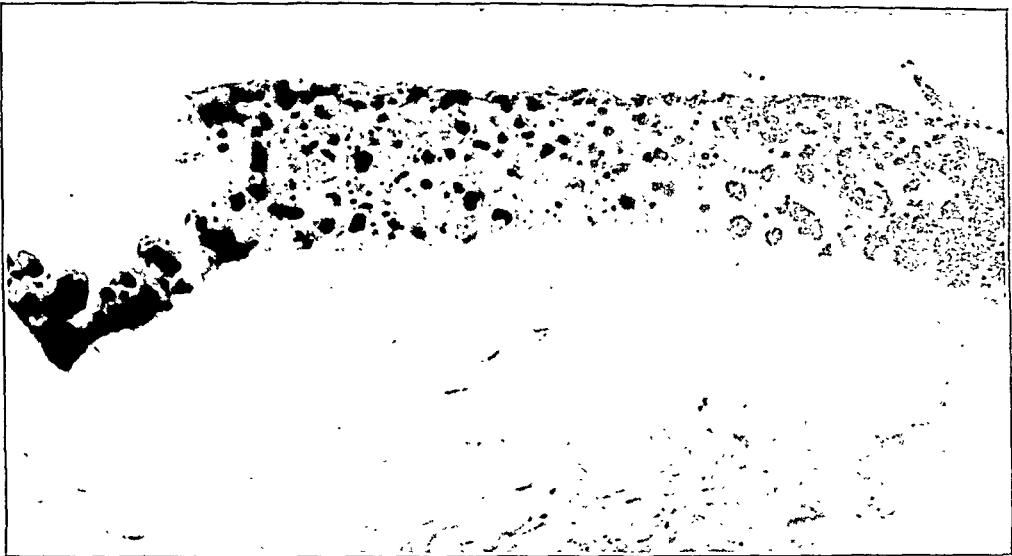
- FIG. 3. Fowl-pox lesion in cornea of chick, seven days after inoculation. The black bodies are the virus bodies, which are stained a brilliant red with the Scharlach R. Frozen section. Herxheimer's stain.  $\times 300$ .
- FIG. 4. Vaccinia lesion in cornea of chick, three days after inoculation. Guarnieri bodies may be seen in three of the cells on the basement membrane. Hematoxylin and eosin.  $\times 1200$ .
- FIG. 5. Higher magnification of one of the cells pictured above. The areola about the Guarnieri body and the dell in the nucleus are clearly shown. Hematoxylin and eosin.  $\times 1900$ .



I



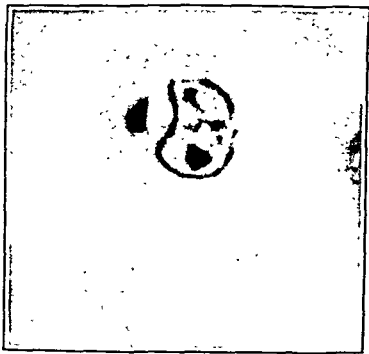




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marked leukopenia, a large number of polymorphonuclear leucocytes are known to be produced rapidly and pass into the circulating blood so that leucocytes known to be in varying stages of the life cycle could be studied. Such a situation is offered in the conditions

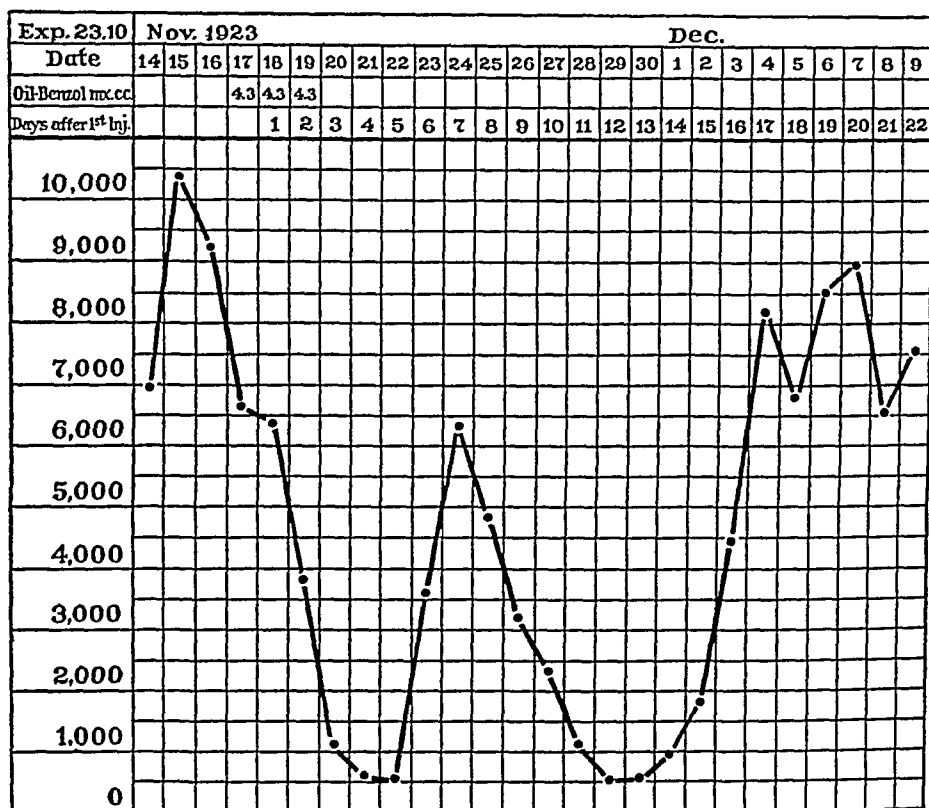


CHART 17 (Exp. 23.10). Total leucocyte curve.

resulting from subcutaneous injections in rabbits of an olive oil-benzol mixture such as have been reported in previous articles of this series.

Article I<sup>4</sup> of this series shows that, as a result of such injections, there is a rapid decrease in the number of leucocytes in the peripheral circulation, after which a primary rise occurs. This is in turn followed, in each case, by a secondary fall and a secondary rise to a normal level, similar to that existing before the injections were made (Chart 17). The term "protophase" was applied to the primary fall and rise and "deuterophase" to the subsequent fall and rise. In articles V<sup>5</sup> and VII<sup>6</sup> of the series it has been further pointed out that the cells chiefly involved in the diphasic leukopenia are

THE VALUE OF THE ARNETH COUNT IN DETERMINING  
THE AGE OF NEUTROPHILE (AMPHOPHILE) LEUCOCYTES  
(RABBIT)\*

THE ACTION OF BENZOL VIII

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In 1904 Arneth published a monograph, "Neutrophile Leucocytes in Infectious Diseases,"<sup>1</sup> based on a study of blood smears from many different cases of acute and chronic infections. He stated that the most important morphological changes were found in the nuclei of polymorphonuclear neutrophile leucocytes. He divided these leucocytes into five different classes according to the arrangement of the nuclear material.

Briefly the classes are: Class I, mononuclear forms of the myelocyte type or with slightly indented nuclei; Class II, those with two distinct nuclear lobes; Classes III, IV, and V, showing three, four and five lobulations of the nuclei respectively. Each class has subdivisions according to the shape of the nuclear portions, whether round or S-shaped. Arneth stated that the percentage of cells in the various classes is constant in health but changes in infectious conditions, the change usually being in the direction of an increase in the percentage of Classes I and II, and decrease or disappearance of cells in Classes III, IV and V. He interpreted these findings by assuming that the simpler nuclei represent an earlier stage of development and in infectious processes the older cells are replaced by those of a younger generation. Particular emphasis has been given to the prognostic value of this classification in pulmonary tuberculosis.<sup>2</sup>

Since Arneth's report various other investigators<sup>3</sup> have studied the question of the nuclear lobulations of the polymorphonuclear leucocytes and several simplifications of the original classification have been made.

Experimental verification of the interpretation of Arneth's classification would seem to be possible in a situation where, following a

\* Received for publication October 21, 1929.

TABLE I  
*Arneth Count (percentages) Exp. 14.38*

Oct. 4, 1915	Total leucocyte count	ARNETH COUNT			
		Unsegmented nuclei		Segmented nuclei	
		Group I	Group II	Group III	Group IV
Average before injection . . . . .	14,000	0	31	38	36
Extreme leucopenia . . . . .	500	21.6	64	7.1	7.1
Deuterophasic rise 1st day . . . . .	600	4	67	20	9
2nd day . . . . .	2,100	1.3	65.6	27	6
5th day . . . . .	9,400	8.7	45.2	28.7	17.3
7th day . . . . .	16,800	3	42	36	19
9th day . . . . .	17,500	2.6	27	32	38

TABLE II  
*Arneth Count (percentages) Exp. 23.10*

Nov. 17, 1923	Total leucocyte count	ARNETH COUNT			
		Unsegmented nuclei		Segmented nuclei	
		Group I	Group II	Group III	Group IV
Average before injection . . . . .	9,000	0	29.9	35.7	34.3
Extreme leucopenia . . . . .	500	*	*	*	*
Deuterophasic rise 3rd day . . . . .	4,460	5.8	60.8	28.9	4.3
4th day . . . . .	8,200	1.7	47.3	35	16
6th day . . . . .	8,500	1.3	44.1	32.4	22
7th day . . . . .	8,980	0	41.9	31.2	26.8
9th day . . . . .	7,540	0	36	32	32

\* Insufficient number of amphophiles found to warrant tabulation.

polymorphonuclear amphophiles and that the phenomenon is accompanied by marked aplasia of the bone marrow following the injections, with active regeneration occurring at the time of the deuterophasic rise. This deuterophasic rise is the result of the entrance into the circulation from the regenerating marrow of newly formed amphophiles.

A study of the forms of the amphophile nuclei during this rapid increase in numbers offers excellent opportunity to test the validity of Arneth's assumption that leucocytes with simple nuclei are young cells and those with segmented nuclei are more mature.

### EXPERIMENTS

The study was made as follows: Smears of blood taken on several days preceding the benzol injections were examined to obtain a basis of comparison and the amphophiles counted and classified as described below. Counts were then made on blood smears taken during the time of the deuterophasic rise and the results tabulated in percentages of the different groups. The blood smears were stained with Wright's stain.

The method used in classifying the amphophiles is based on a report by Pons and Krumbhaar,<sup>3</sup> the cells being arranged in four groups as follows:

*Group I:* Cells, the nuclear material of which is in one mass, round, oval or indented not more than one-half through its width.

*Group II:* Cells, the nuclear material of which is not divided into segments, but may be lobed, spiral, looped, rosette or variously irregular.

*Group III:* Cells, the nuclear material of which shows segmentation into *two parts* either entirely separate (as viewed in the smear) or seen to be connected by a narrow filament.

*Group IV:* Cells, the nuclear material of which shows segmentation (as in Group III) into *more than two parts*.

The reason for separating Groups III and IV is that some added information is thus given and a clearer conception may thus be formed of the numerical status of the (so-called) oldest cells. Also, Group III seemed to form a sort of "middle group" which varied less with changing conditions than the extremes in I and IV.

Eight cases and approximately ten thousand cells were studied. Cells which were badly distorted or poorly stained were not counted.

2. Cells of Group II are the most prominent type at the beginning of regeneration and in turn decrease to a percentage comparable to that of the average before injection.

3. Cells of Group IV are relatively rarer in the early days of regeneration and increase in number until the average relation before injection is approximated.

### CONCLUSIONS

The cells liberated from a regenerating bone marrow in such quantities as to produce a rapid increase in the total number of circulating leucocytes must of necessity have a relatively larger number of recently formed cells than would normally be found. Such rapid increase means that new cells are entering the circulating blood more rapidly than those already present mature and end their life cycle.

Normally the production and death of amphophiles must proceed at a fairly uniform rate and the percentage of young and old forms would be expected to remain fairly constant through this balance of production and destruction of the cells. The counts made on normal animals showed that the Arneth count remains constant within reasonable limits. When the total number of leucocytes is rapidly changing, the balance of production and death of cells is obviously disturbed. Such a condition resulted from the benzol injections and, in the portion of the curve studied, intensive production was taking place causing "increasing birth rate over death rate." At this point a definite increase in the percentage of single lobed nuclei over multilobed nuclei was found to take place.

The conclusion, therefore, seems justified that amphophiles with single lobed nuclei are the young or immature forms and that the number of lobes increases with maturity of the cells.

We believe, therefore, from these studies that the Arneth count made from smears from the blood of the rabbit is of definite value in determining the relative age of the amphophiles, and that a "shift to the left" in the count (increase in per cent of the simpler formed nuclei) actually indicates a relative increase in the number of young or more immature amphophiles in the circulating blood.

TABLE III  
*Arneth Count (percentages) Exp. 23.11*

Nov. 17, 1923	Total leucocyte count	ARNETH COUNT			
		Unsegmented nuclei		Segmented nuclei	
		Group I	Group II	Group III	Group IV
Average before injection .....	8,000	0	31	35	34
Extreme leucopenia .....	200	*	*	*	*
Deuterophasic rise 2nd day .....	1,000	**	**	**	**
3rd day .....	3,000	9.5	61.9	21.4	7.1
5th day .....	6,800	6.4	48.9	21.7	22.8
7th day .....	7,800	3.7	42	35.5	18.7
10th day .....	9,600	0	40	37	23

\* Insufficient number of amphophiles found to warrant tabulation.

\*\* Smear unsatisfactory.

In making the counts at the stage of most extreme leucopenia, a search of the entire slide was made and even then in some instances only eighteen to twenty amphophiles were found. However, as the character of these cells at this point was fairly uniform and clear-cut in every case, the results were felt to be sufficiently accurate to be acceptable.

Some of the slides did not show the nuclear divisions clearly. The counts on these days were omitted. The results in general were uniform in all cases studied. Those in three cases are shown in Tables I, II, and III.

## RESULTS

The tables show that, in the period corresponding to the regeneration of the bone marrow and consequent liberation of amphophiles into the circulating blood (*i. e.* deuterophasic rise), the following variations from the normal averages occur.

1. Cells of Group I appear in the circulation in percentages varying from 2 per cent to 21 per cent, then gradually drop out of the blood picture as the leucocyte count returns to normal. The counts made before the benzol injections show no cells of this type.



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## HISTOPATHOLOGY

As a basis for the subsequent discussion it is proposed to consider briefly at this time those features of the histopathology of the marrow which have to do with the diphasic character of the leucopenia.

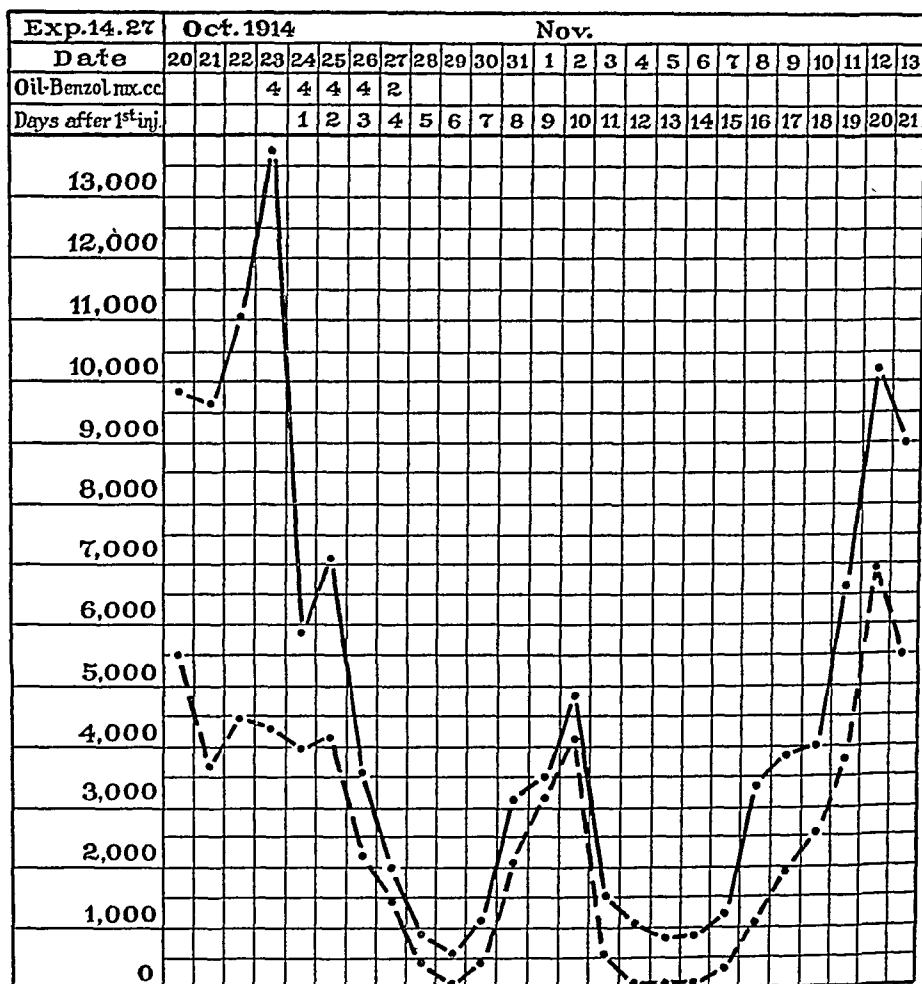


CHART 18 (Exp. 14.27). Total leucocyte and absolute polynuclear amphophile curves.

The histopathology is based on rabbits which died and on rabbits which were killed at various stages of the diphasic leucopenia and showed typical though incomplete diphasic leucopenia curves up to the time of death. For these reasons the histopathological pictures cannot be perfectly correlated with complete blood pictures be-

# THE NORMAL LIFE SPAN OF THE NEUTROPHILE (AMPHOPHILE) LEUCOCYTE (RABBIT)\*

## THE ACTION OF BENZOL IX

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Syracuse, N. Y.)*

Heretofore apparently the life span of somatic cells in their normal somatic environment has not been determined accurately. This article establishes with reasonable accuracy the normal length of life in the body of the normal amphophile leucocyte from the time when it passes from the marrow into the blood and under somatic conditions which are relatively normal.

In previous articles of this series<sup>1, 2</sup> attention was called to the fact that after exposure to the action of subcutaneous injections of olive oil-benzol mixture (equal parts of olive oil and benzol), the leucocyte curve follows a course, a portion of which we have called "diphasic leucopenia." In an average of six days after the beginning of an average of four and a half daily injections of 1.5 to 2 cc. per kilo of body weight, the curve falls to the neighborhood of 1,000. This fall is followed by a primary rise to a normal level. Then, independently of any further injections, the curve again falls to a level as low or nearly as low as reached in the primary fall. Following this secondary fall there occurs a rise of the curve to a normal level and there is no evidence of any subsequent falls (Charts 18 and 19). To the phase of the leucocyte curve involving the first period of leucopenia we have given the name "protophase," and to that involving the second period of leucopenia the name "deuterophase."

In the fifth article<sup>3</sup> of this series it was shown that in the diphasic leucopenia resulting from subcutaneous injections of olive oil-benzol mixture in rabbits the amphophile and small mononuclear curves run more or less independently of each other and that the diphasic character of the leucopenia is essentially a polynuclear amphophile phenomenon.

\* Received for publication December 8, 1929.

The injections of the benzol mixture result in necrosis and aplasia of the bone marrow. In surviving animals this is followed by resolution and regeneration. Regeneration is initiated by the development from various centers of islands of regenerating cells. These islands consist of practically pure cultures of the various bone marrow cells. In the early stages of regeneration these centers are well isolated, some consisting entirely of myelocytes (Figs. 1 and 2), others of megakaryocytes (Figs. 3 and 4), and still others of nucleated red cells. Later as regeneration progresses the various centers tend to intermingle in their growth and are accompanied by the mature cells which they produce. Observations bearing upon this matter were made by Selling,<sup>4</sup> and have been confirmed by MacCallum.<sup>5</sup>

In relation to the diphasic amphophile leucopenia, we have confined the marrow studies to amphophiles and amphophilic myelocytes, these latter being perhaps the only cells of the marrow parenchyma that can be certainly identified in fixed and stained tissues as being forerunners of amphophiles in the blood. In the sections we distinguish between original and regeneration myelocytes. As mentioned above the regeneration myelocytes appear to be characteristic in morphology and environment.

The regeneration myelocytes make their earliest appearance at the climax of the deuterothymic phase; the earlier ones probably differentiating from more immature forms.

All other amphophilic myelocytes in the marrow are like those in normal marrow. For this reason we designate them as original myelocytes.

Examination of the marrows of rabbits dying or killed at the climax of the protophase, shows marked necrosis and aplasia. However, practically all show certain numbers of amphophiles and original amphophilic myelocytes but no regeneration myelocytes. As will be emphasized later, it is these surviving amphophiles and surviving original myelocytes which bring about the protophasic rise.

The marrows of rabbits killed at the end of the protophasic rise show practically complete aplasia with very few, if any, amphophiles or original amphophilic myelocytes, and no regeneration myelocytes. The marrow appears practically completely aplastic.

The marrows of rabbits dying and killed at the climax of the



the blood and marrow has discontinued, because return of the urinary phenol curve to the level existing before injections indicates that the injected benzol has been eliminated.<sup>6</sup> The end of the protophasic amphophilic rise occurs about two days later. The protophasic amphophilic rise is the result of access from the marrow to the blood of surviving mature amphophiles and of amphophiles differentiating from surviving original amphophilic myelocytes. The two processes of access and differentiation may be assumed to go on more rapidly with the cessation of the action of injected material on the involved cells. The rapidity would probably be accentuated as a reactive result of the existing leucopenia.

Examination of the average amphophile curve in Chart 9,<sup>3</sup> shows that the amphophile level at the end of the protophasic amphophilic rise is the same as that existing before injections. Examination of the amphophile curve in Chart 19 shows that it may remain at this level for over four days. This period of constant level which occurs in a considerable number of cases is of fundamental interpretative importance. During this period elevations and depressions in the curve are due to daily variation in the amphophile count. In cases in which there occurs such a period of constant normal level at the end of the protophasic rise it means that the supply of marrow amphophiles (from original myelocytes) available to the blood is large enough, not only to bring about the protophasic amphophilic rise to the level existing before injections, but also to maintain it at that level for a time by compensating for the disappearance from the blood of those which perish as a result of reaching the end of their life history.

In cases in which no such period of constant level (Chart 18) occurs it means that the supply of marrow amphophiles available to the blood is exhausted in bringing about the protophasic amphophilic rise. As a matter of fact such supply of amphophiles is in some cases insufficient to bring the protophasic rise up to the original normal level.

Next, the exhaustion of the supply of marrow amphophiles available to the blood results in the deuterophasic amphophilic fall. The deuterophasic amphophilic fall is directly dependent upon the disappearance from the blood of those amphophiles which perish.

At the beginning of the fall, practically the total potential supply of amphophiles is represented by those in the blood. Among them

deuterophase show extreme aplasia with here and there colonies of regenerating cells such as described above. The sections indicate active regeneration occurring in practically completely aplastic marrows.

The marrows of rabbits killed some time after the completion of the cycle apparently differed in no way from the marrow of rabbits which had not received injections.

### THE AMPHOPHILE CURVE

I propose now to examine certain aspects of the amphophile curve and to give a correlative interpretation with certain prominent features of the histopathology of the marrow. Perhaps the most important result will be to show that low levels of the amphophile curve are apparently entirely dependent upon two factors, either one sometimes acting alone, or at other times both acting together:

1. The extent of the destruction or death of amphophiles going on in the blood.
2. The number of amphophiles in the marrow available to the blood.

This will mean that low levels are independent of any alteration in the potential balance of physicochemical or other biological reactions, such as amphophilotropism. The amphophile curve remains at or below the normal level depending upon the available supply of amphophiles.

As illustrated in Charts 18 and 19, there is a period of about two days after the beginning of injections and before the beginning of the protophase, during which the amphophile curve shows no effect of the injections. During this period, if there is any destruction of amphophiles in the blood, it is at once compensated for by access of marrow amphophiles into the blood.

During the protophasic amphophilic fall, destruction of amphophiles in the blood cannot be fully compensated for on account of the effective amphophile poverty of the marrow. This accounts for the occurrence of the protophasic amphophilic fall.

The first evidence of the protophasic amphophilic rise appears about at the end of the third day after the last injection. At this time it is likely that initiation of amphophile destruction, that is, attack upon additional amphophiles and amphophilic myelocytes in

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## DESCRIPTION OF PLATES

### PLATE 39

FIG. 1. Bone marrow regeneration. Island of regenerating myeloblasts (Exp. 14.34).  $\times 85$ .

FIG. 2. Same as Fig. 1.  $\times 1100$ .

all ages are represented. At the end of the fall they have nearly all disappeared. This means that the youngest of them disappear within this period. The maximum duration of life of nearly all of the amphophiles in the blood is thus not more than the interval between the beginning and the end of the deuterophasic fall. The average duration of this interval computed from twenty-six cases of diphasic leucopenia is three and a half days.

Perhaps the main complicating factor in connection with this argument is the increasing amphophile leucopenia which exists during the fall. This may be conceived of as placing an abnormal functional strain upon the amphophiles present in the blood and thus shortening their lives. Nevertheless the conditions approach vastly more nearly normal than those involved in *in vitro* studies of this problem. I know of no proof that such unusual functional cell activity shortens the lives of somatic cells.

The initiation of the deuterophasic amphophilic rise depends on access to the blood of marrow amphophiles differentiating from regeneration as distinguished from original amphophilic myelocytes. As the supply gradually increases with progressive amphophilic marrow regeneration, the rise continues until the level existing before injections is reached, as in the case of the protophasic amphophilic rise, and then this level is maintained.

### CONCLUSIONS

1. Cessation of the supply of amphophiles from the marrow results in practically complete disappearance of amphophiles from the circulating blood in a period of between three and four days.
2. The average duration of life of amphophiles in the rabbit's blood is between three and four days.

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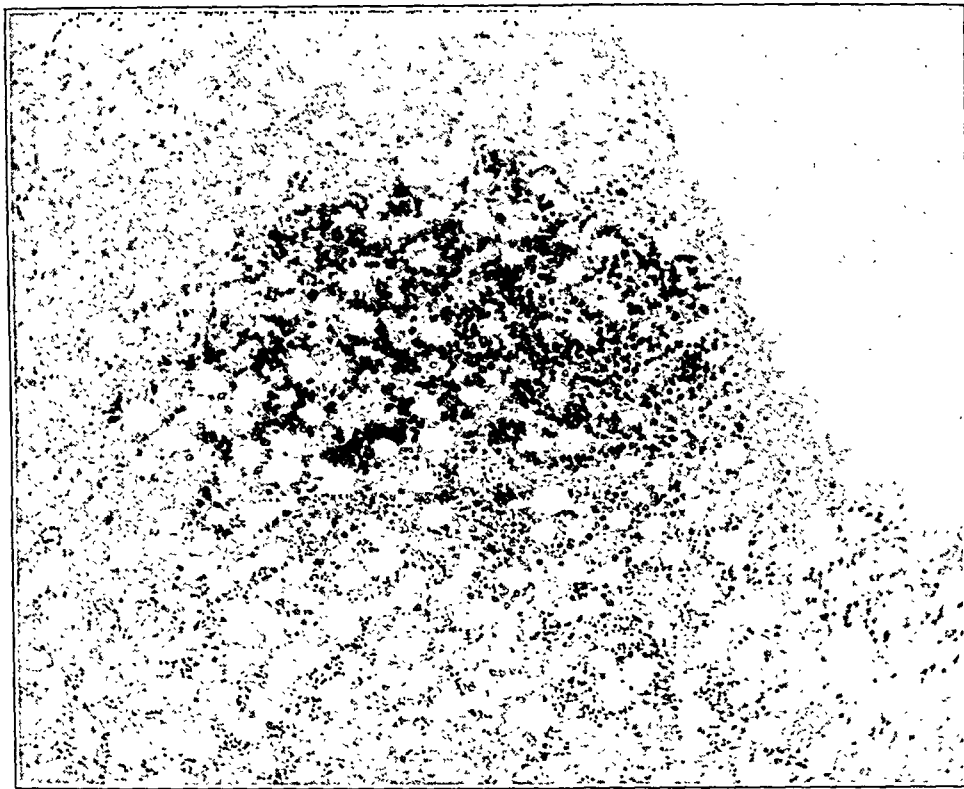
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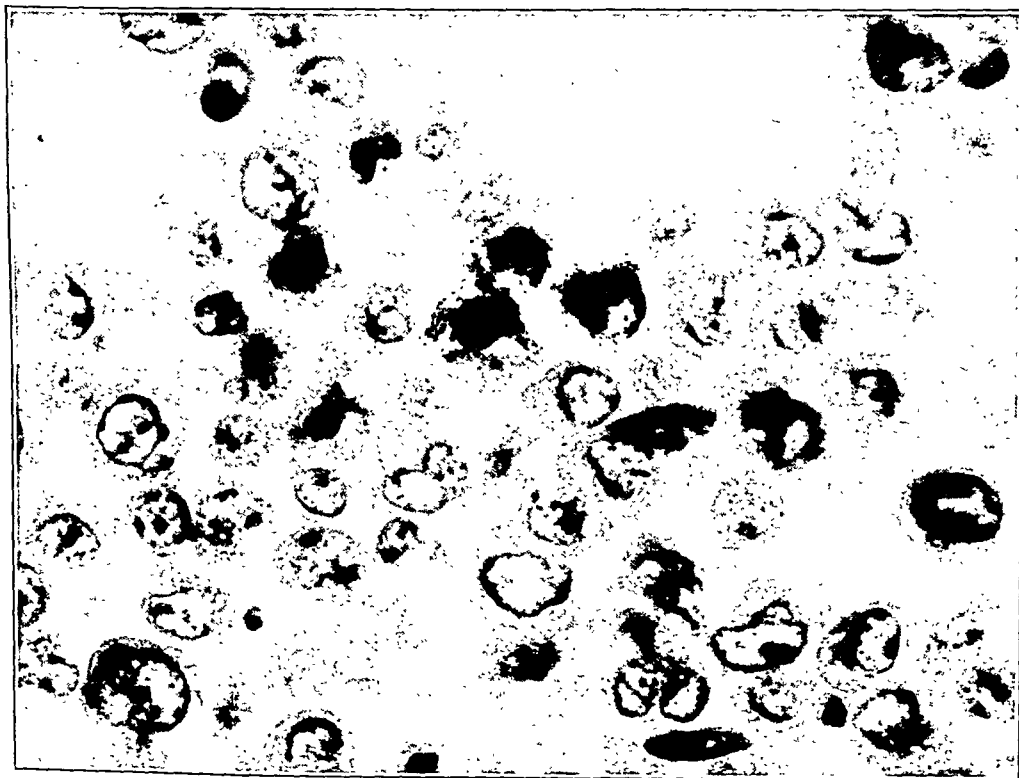
PLATE 40

FIG. 3. Bone marrow regeneration. Island of regenerating megakaryocytes  
(Exp. 14.34 same section as Fig. 1).  $\times 85$ .

FIG. 4. Same as Fig. 3.  $\times 1100$ .

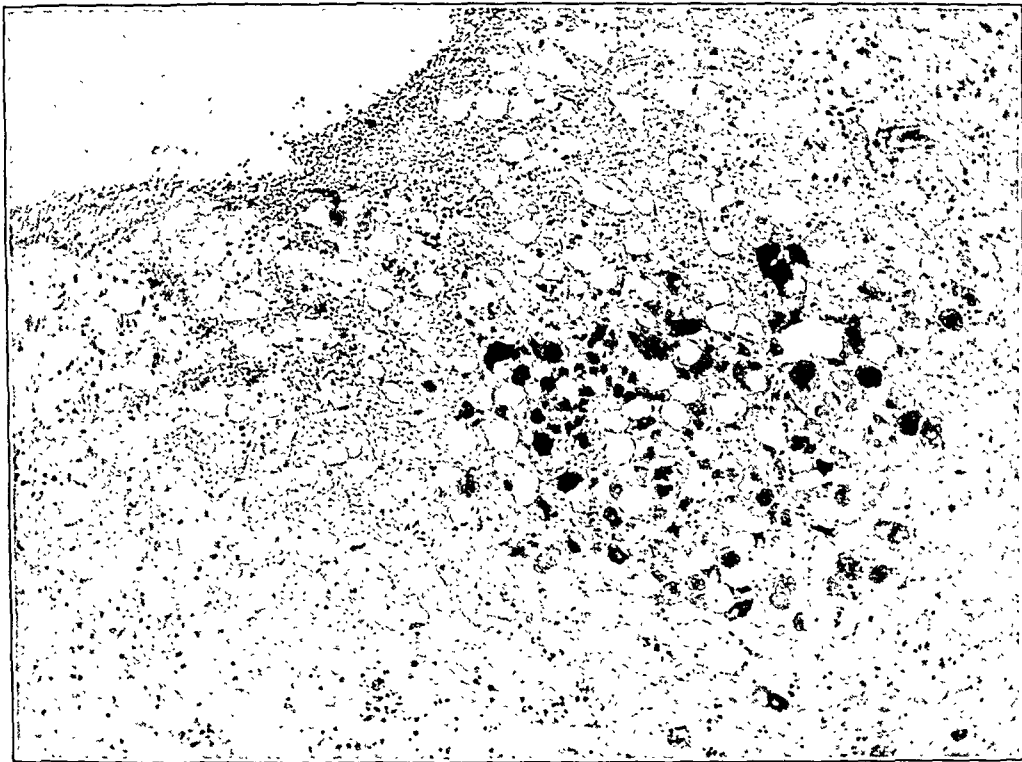


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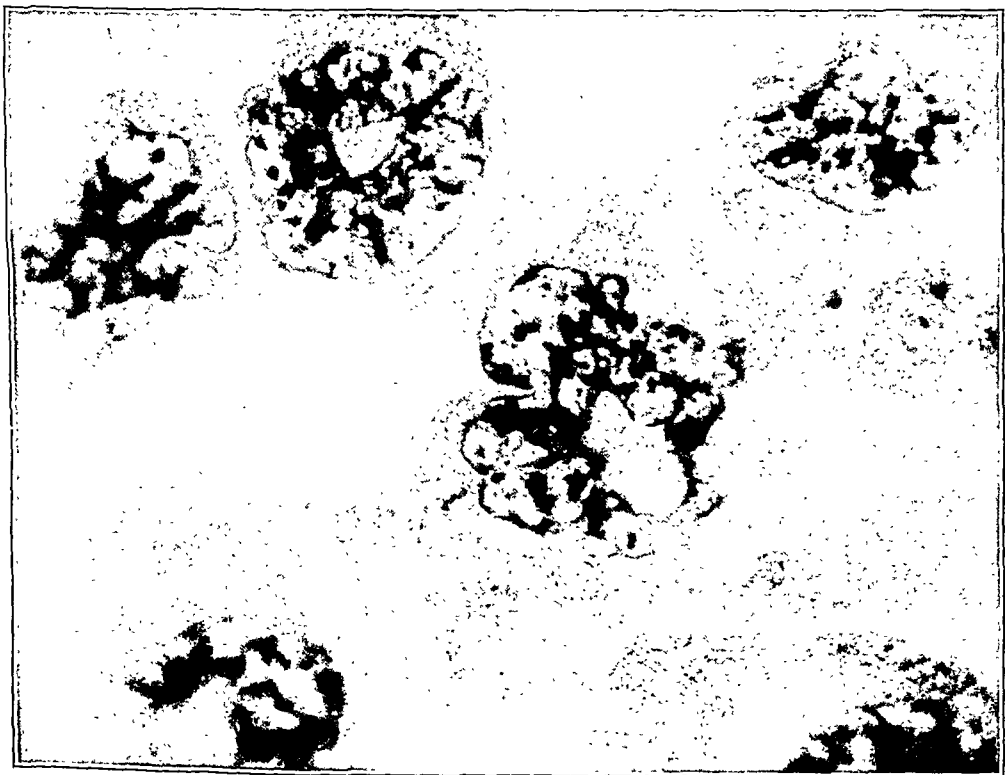


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3



4

sinistra exactly as would the vena azygos into the normal superior vena cava (Figs. 2 to 4). No trace or vestige of a right vena cava superior was found nor was there a vena azygos on this side (Fig. 4). No other deviation of significance was found in the remaining tributaries of the superior vena cava. The course of the inferior vena cava and its tributaries was normal.

#### COMMENT

The presence of a vena cava superior on the left side as well as on the right is the normal condition in certain animals. Such an arrangement of the great venous trunks in man is rather infrequent. If present, the anomaly may be looked upon as persistence of a condition existing only during the latter part of embryonic, and the beginning of fetal life.<sup>1,2</sup> Of this anomaly well over a hundred cases have been reported up to 1916<sup>3</sup> and undoubtedly many more observed.<sup>4</sup> The presence of a left vena cava superior without a right vena cava superior is a far more spectacular condition and one less likely to be overlooked. Yet in the available literature to date there appear to be records of only about seventeen cases.

According to Krause,<sup>5</sup> Cheselden<sup>6</sup> reported the first case. The complete report, however, printed in the Philosophical Transactions of the Royal Society of London in 1713 reads as follows: "A heart, with the vena azygos inserted into the right auricle; and the descending cava coming round the basis of the heart, above the aorta and pulmonary vessels, to enter the auricle at the lower part with the ascending cava." The brevity of this description leaves some doubt as to the existence of an actual situs inversus of the superior vena cava with complete absence of the right vena cava superior.

The first detailed description of a case in which the superior vena cava showed a complete situs inversus was given by Halbertsma<sup>7</sup> in 1862. His observations were made on an injected heart, apparently of an adult. The two innominate veins united in front of the arch of the aorta to form the superior vena cava which ran from above downward and to the left, thence continuing in front of the pulmonary artery, and between the pulmonary veins and left auricle it reached the coronary sulcus and passing to the right emptied into the right atrium below and to the left of the inferior vena cava. The vena azygos emptied into the left vena cava superior 4 cm. below the union of the innominate veins.

## COMPLETE SITUS INVERSUS OF THE VENA CAVA SUPERIOR \*

BÉLA HALPERT AND FRANCIS D. COMAN

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During the routine anatomical investigation of the body of a 2 weeks-old, well nourished, male, negro infant, a vascular anomaly was found which may be termed a "complete situs inversus of the vena cava superior," the arrangement and course of the dural sinuses, the large venous trunks of the neck, the vena cava superior and the vena azygos being a mirror image of the normal. The drawings by Mr. J. F. Didusch illustrate the condition.

At the confluens sinuum the sinus sagittalis superior appeared to continue into the left sinus transversus (Fig. 1), while the sinus rectus continued into the right sinus transversus. No difference was noted in the size of the internal jugular veins. On the right as well as on the left side, the vena jugularis interna joined the vena subclavia in the usual manner. The right vena anonyma, however, had the usual length of a left vena anonyma, measuring about 25 mm. in this case and appearing rather a continuation of the vena subclavia dextra than that of the vena jugularis interna dextra. The left vena anonyma had the usual length of a right vena anonyma, measuring about 9 mm., and appearing rather a continuation of the vena jugularis interna sinistra than of the vena subclavia sinistra (Fig. 2).

The junction of the right and left venae anonymae occurred in front of the arch of the aorta at about the level of the left sterno-clavicular articulation. The vena cava superior, thus formed, joined the sinus coronarius after passing between the pulmonary artery and the left auricle on its right and the upper lobe of the left lung to the left. The size of the vena cava superior sinistra was about the same as that of a normal right vena cava superior. It measured from the anonyma angle to the point of entrance into the sinus coronarius about 30 mm.; the length of the greatly enlarged coronary sinus was about 25 mm. (Fig. 3). Before entering the pericardial cavity, a large venous trunk emptied into the vena cava superior

\* Received for publication November 18, 1929.

In 1899 Mäusert<sup>13</sup> described a complete situs inversus of the vena cava superior in a 4 year-old girl, dead from diphtheria. The right vena cava superior was entirely absent and the left vena cava superior emptied into the greatly enlarged coronary sinus which in turn opened into the right atrium, a little more to the left than is usual. The heart was otherwise normal. There were two azygos veins: a larger left emptied into the vena cava superior just before it entered the pericardial sac, a narrow right emptied into the right innominate vein.

In 1910 Gozuloff<sup>14</sup> described a complete situs inversus of the vena cava superior in a 30 year-old male typhus victim. The anomaly was noted six years previously by V. P. Oven in the course of routine dissection in the Department of Anatomy, University of Kiev. No trace of a right vena cava superior was present. The vena azygos emptied into the left superior vena cava 5.5 cm. below the junction of the innominate veins. The vena hemiazygos emptied into the vena azygos.

Dietrich<sup>15</sup> in 1913 described a complete situs inversus of the superior vena cava in a 29 year-old woman who died of fibroxanthosarcomatosis. There was no vena azygos on the right side. The left vena azygos entered the superior vena cava just before it pierced the pericardium.

Schütz<sup>16</sup> in 1913 reported a clear-cut complete situs inversus of the vena cava superior in a male, aged 61 years. "Vena cava superior in latere sinistro descendens (ductus Cuvieri sinister persistens). Vena azygos sinistra, vena hemiazygos dextra." This case had been recorded previously by Genersich in the Hospital Annals of the City of Budapest in 1910. Schütz mentions an exactly similar case noted in the First Pathological Institute of the University of Budapest.

Nützel<sup>17</sup> in 1914 described a case, in a 25 year-old male, in which the only vena cava superior present was the left. This opened into the coronary sinus at the atrioventricular border line. The coronary sinus was large and although emptying into the left atrium communicated also with the right. At the junction of the superior vena cava and the coronary sinus emptied the vena cordis magna "*linke Kranzvene*." The vena azygos opened into the vena cava superior sinistra.

Smith<sup>18</sup> in 1916 described a complete situs inversus of the vena

In 1876 Greenfield<sup>8</sup> reported to the Pathological Society of London a case of a male, aged 39 years, in which due to an abnormal mode of development of the great venous trunks the left duct of Cuvier instead of the right, persisted as the main channel for the venous blood from the head and upper limbs to the right atrium. In this case apparently the left trunk alone persisted; "the right, if present, which is doubtful, being represented by only a very small vein: the usual condition being thus reversed, and the whole of the blood from the upper extremities and head entering the coronary sinus. . . . The heart was unfortunately removed before the relation of the great vessels was traced."

In 1880 Gruber<sup>9</sup> described the transposition of the normal relationship of the innominate veins in an adult male, *i. e.*, the vena anonyma dextra was the longer of the two and ran obliquely downward to the left. The innominate veins united to form a left vena cava superior. There was no trace of a right vena cava superior. A vena intercostalis suprema, a vena hemiazygos superior, and a vena hemiazygos inferior were found on the right side; a vena intercostalis suprema and a vena azygos were noted on the left side.

The persistence of a vena cava superior sinistra and the absence of the normal right vena cava superior in a man 26 years old, described by Weigert<sup>10</sup> in 1881, was attributed to an early synostosis of the sutura mastoidea, aplasia of the right transverse sinus and of the right internal jugular vein. The superior sagittal sinus continued into the left transverse sinus. The jugular foramen on the right was only one-third the size of that on the left.

The next undoubted case of complete situs inversus of the vena cava superior was presented in 1892 by Bédart<sup>11</sup> before the Society of Anthropology of Paris. No remnant of a right vena cava superior was present. The author gives only a meager description and apologizes for presenting the case which according to him represented a condition frequently reported previously. No references, however, are given.

In 1893 Boyd<sup>12</sup> demonstrated before the Anatomical Society of Great Britain and Ireland a complete situs inversus of the superior vena cava in a fetus. No trace of a right vena cava superior or duct of Cuvier could be found. There were two azygos veins of equal size: the right emptied into the right innominate vein, the left into the left vena cava superior.



## SUMMARY

A rare vascular anomaly in a 2 weeks-old negro infant\* is described. The arrangement and the course of the dural sinuses, the large venous trunks of the neck, the vena cava superior and the vena azygos presented a mirror image of the normal. There was no trace of a right vena cava superior.

Previous reports of this anomaly are reviewed.

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\* A brief description of this case appeared in the issue of the *Anatomical Record*<sup>23</sup> which contains the abstracts of papers presented at the forty-third session of the American Association of Anatomists.

cava superior in a middle-aged male. There was no trace of a right vena cava superior. The vena cava superior sinistra emptied into the coronary sinus which joined the inferior vena cava at its entrance into the right atrium.

Mönckeberg<sup>19</sup> (1924) found in the collection of the Pathological Institute of Tübingen, the heart of an adult which apart from the persistence of the left vena cava superior and absence of the right, presented no other developmental or pathological changes.

Benda<sup>20</sup> (1924) mentions a complete situs inversus of the vena cava superior in an adult male, and illustrates the case with diagrams.

Villa<sup>21</sup> (1926) working apparently on an adult body noticed the presence of a left vena cava superior which continued into the sinus coronarius. He found no trace of a right vena cava superior; the vena azygos emptied into the left vena cava superior.

## DISCUSSION

In terms of embryology the vena cava superior consists: (*a*) of a proximal portion which is identical with the duct of Cuvier, and (*b*) of a distal portion formed by the terminal part of the vena cardinalis superior. The cardinal portion of the superior vena cava begins at the junction of the innominate veins, its transition into the Cuvierian portion is marked, of course, by the entrance of the vena azygos which represents the cranial half of the vena cardinalis inferior. In our case the right superior vena cava either has not developed or else has disappeared entirely, while the left remained taking functionally the place of the right. Whether the entrance of the sinus sagittalis superior into the left sinus transversus had anything to do with this transposition or whether it is only a part of it is open to discussion.<sup>22</sup> It may be recalled that the course of the sinus sagittalis superior is mentioned only by Weigert.<sup>10</sup> In his case, as in ours, the sinus sagittalis superior continued into the left sinus transversus. Weigert attributed the presence of the left vena cava superior to an early synostosis of the sutura mastoidea, aplasia of the right sinus transversus and of the right vena jugularis interna.

## DESCRIPTION OF PLATES

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### PLATE 41

FIG. 1. View of the confluens sinuum. The sinus sagittalis superior enters the left sinus transversus.

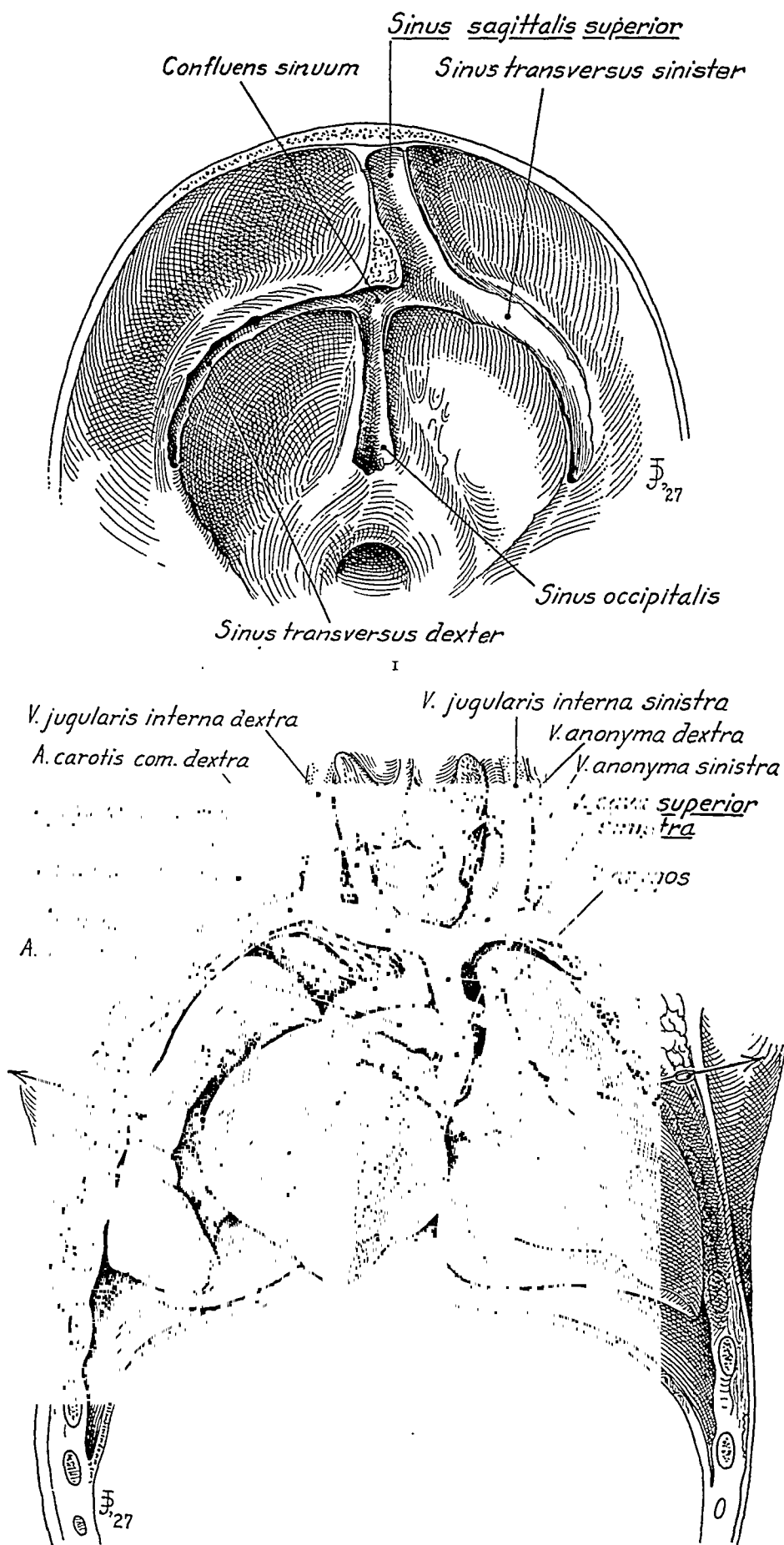
FIG. 2. Arrangement of the large vessels of the neck and thorax. The arrangement and course of the large venous trunks of the neck, the vena cava superior and the vena azygos present a mirror image of the normal. The right vena anonyma has the usual length of a left vena anonyma, while the left vena anonyma has the usual length of a right vena anonyma. By the junction of the right and left venae anonymae a left superior vena cava is formed which passing in front of the aorta and arteria pulmonalis joins the sinus coronarius. The vena azygos enters the vena cava superior sinistra as would the normal azygos vein the normal superior vena cava.

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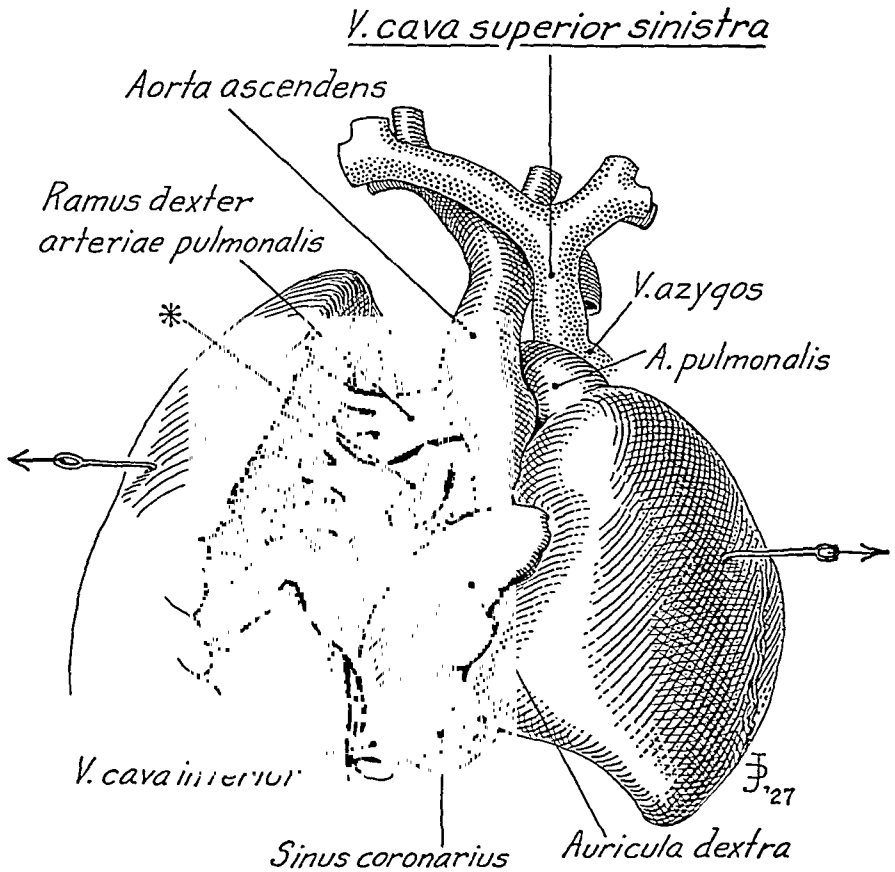
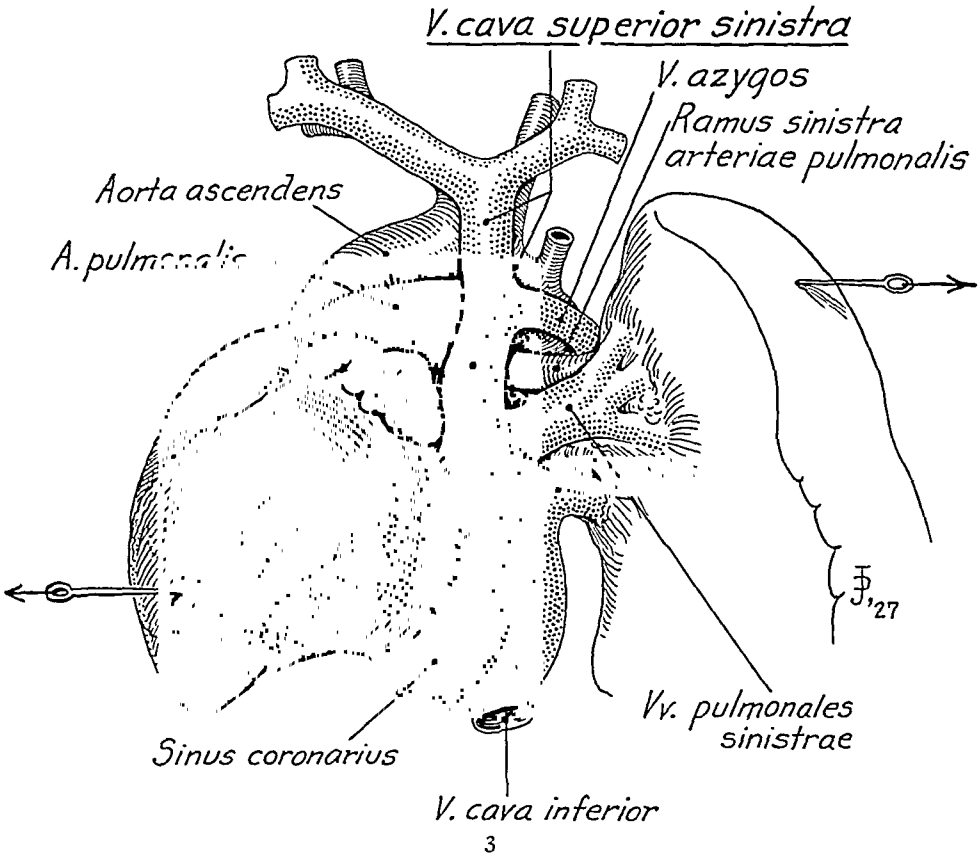
PLATE 42

FIG. 3. Entrance of the vena cava superior sinistra into the sinus coronarius.

FIG. 4. View of the right atrium. The asterisk marks the site where the vena cava superior normally enters the right atrium.









sium ferrocyanide. The general principles followed in injection are given by Scott and Moore.<sup>8</sup> When the entire circulatory tree has been filled with this solution the kidney is cut into eight to ten sagittal sections and the cortex carefully separated from the medulla. The cortex is placed in 25 per cent hydrochloric acid for twenty-four hours, when it is washed in water and weighed.

Duplicate or triplicate samples are removed, and separately weighed. Each sample consists of ten to twelve small pieces from different portions of the kidney. It is planned that these samples together shall constitute from one twentieth to one fiftieth of the total weight of the cortex. The samples are replaced in 25 per cent hydrochloric acid and allowed to digest until the tissue just falls apart on gentle pressure with a needle, when they are washed and suspended in a mixture of 3 parts glycerol and 1 part 10 per cent chloral hydrate. The tissue is emulsified in the glycerol and placed on slides with a pipette. The glomeruli are plainly visible with a 32 mm. objective and  $\times 5$  or  $\times 10$  eyepiece and are enumerated with the aid of a mechanical stage. From the count of the total glomeruli in the weighed sample, the number per gram and the total in the entire kidney is easily calculated. At the time of removal of samples for counting, five to six specimens were removed, fixed in 10 per cent formalin and sectioned. A study of these sections served as a check on the completeness of injection. In all cases at least 96 per cent of the glomeruli have shown Prussian blue within the capillary loops.

A detailed study of the errors of this method of enumeration of the glomeruli in the human kidney will appear in conjunction with studies of the normal kidney from this laboratory.

## RESULTS

Three cases of enlargement associated with hypoplasia in man and one case of agenesis in a dog have been studied. In each case the glomeruli in the enlarged kidney were enumerated. In one instance a count of the hypoplastic kidney was made. In all cases the kidneys studied have been free of any chronic changes which might be associated with a decrease in number of glomeruli.

# THE TOTAL NUMBER OF GLOMERULI IN THE CONGENITALLY ASYMMETRICAL KIDNEY\*

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In a recent communication<sup>1</sup> the results of total glomerular counts in the enlarged kidney induced by unilateral nephrectomy have been presented. The present paper gives the counts of the total number of glomeruli in congenitally asymmetrical kidneys.

Investigations by others have been limited to a count of the total glomeruli in equal areas of sections from normal and abnormal kidneys, but the results have been variable.

Eckardt<sup>2</sup> assumed that the volume of any two kidneys is in direct proportion to the total number of glomeruli. On the basis of this hypothesis he studied three cases of agenesis in man and concluded that, in congenital defects, there is a true unit hyperplasia, "*eine Vermehrung — sowohl der glomeruli wie der Harnkanälchen*," in the solitary kidney.

F. C. Moore<sup>3</sup> and Gladstone<sup>4</sup> have investigated instances of agenesis in man and conclude, on the basis of relative counts, that there is no increase in the total number of glomeruli in the single enlarged kidney. Boycott<sup>5</sup> studied a case of agenesis in a rabbit and arrived at the same conclusion.

Numerous other workers have studied examples of agenesis and hypoplasia, in most instances from a purely anatomical standpoint. The literature is well summarized by Fortune.<sup>6</sup>

The recent development of an accurate method for enumeration of the glomeruli by Vimtrup<sup>7</sup> has opened a more exact method of approach to this problem.

## METHOD

The general method of Vimtrup was followed. As used by us, it consists of irrigation of the vascular bed of the kidney with tap water, and injection with a medium composed of equal parts of a 1.5 per cent aqueous solution of ferric ammonium citrate and potas-

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TABLE III

KIDNEY No. 52. *Lakeside Hospital No. 3190. Male, 53 years of age, white.*  
*Enlarged, associated with congenital hypoplasia of the opposite kidney.*

Total cortex.....200.2 gm.  
 Medulla.....66.75 gm.

RATIO 1:3

Sample	Weight in grams	Count	Glomeruli per gram
1 .....	4.65	16,800	3,612
2 .....	4.27	14,637	3,428

Total weight samples counted.....8.92 gm.  
 Glomeruli in samples.....31,437  
 Glomeruli per gram.....3,523  
 Estimated total glomeruli in kidney.....705,704

TABLE IV

KIDNEY No. 108. *City Hospital No. 5830. Male, 42 years of age, white.*  
*Enlarged, associated with congenital hypoplasia of the opposite kidney.*

Total cortex.....177.5 gm.  
 Medulla.....42.55 gm.

RATIO 1:4.07

Sample	Weight in grams	Count	Glomeruli per gram
1 .....	3.01	23,336	7,752
2 .....	2.50	18,707	7,482

Total weight samples counted.....5.51 gm.  
 Glomeruli in samples.....42,043  
 Glomeruli per gram.....7,630  
 Estimated total glomeruli in kidney.....1,354,432

TABLE I

KIDNEY NO. 42. *City Hospital No. 5726. Male, 60 years of age, white. Hypoplasia (for enlarged male see Kidney No. 43)*

Total cortex.....27.43 gm.  
Medulla.....20.21 gm.

RATIO 1:1.27

Sample	Weight in grams	Count	Glomeruli per gram
1 .....	0.94	12,417	13,210
2 .....	0.79	11,248	14,238

Total weight samples counted.....1.73 gm.  
Glomeruli in samples.....23,665  
Glomeruli per gram.....13,679  
Estimated total glomeruli in kidney.....375,214

TABLE II

KIDNEY NO. 43. *City Hospital No. 5726. Enlarged — (For hypoplastic male see Kidney No. 42)*

Total cortex.....123.5 gm.  
Medulla.....44.3 gm.

RATIO 1:2.87

Sample	Weight in grams	Count	Glomeruli per gram
1 .....	0.37	2,783	7,521
2 .....	0.075	610	8,133
3 .....	1.19	9,213	7,742
4 .....	1.65	12,606	7,640

Total weight samples counted.....3.285 gm.  
Glomeruli in samples.....25,212  
Glomeruli per gram.....7,674  
Estimated total glomeruli in kidney.....947,739

kidney. This points to a fault of development not associated with a deficient available blood supply, and the condition should be termed a hypoplasia in distinction to a hypotrophy or atrophy. The lesser number of glomeruli also favors the application of the term hypoplasia. The enlarged kidney found in congenital hypoplasia of the opposite side, has been designated "hypertrophic," but it is thought that until there is proof that, in addition to an increased size, there is an increase of functional ability, the term "enlarged" should be employed.

### CONCLUSIONS

The enlarged kidney associated with hypoplasia or agenesis of the opposite kidney contains the usual number of glomeruli characteristic for one kidney of that species.

NOTE: I wish to thank Dr. Alan Moritz and Dr. Otto Saphir for the specimens used in this investigation and Dr. Howard T. Karsner for aid in the preparation of the paper.

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TABLE V

KIDNEY NO. 105. *Dog-Agenesia. (Male, setter type of mongrel, weight 15 Kgm. The thoracic and abdominal viscera were normal with the exception of the right urinary tract. The kidney, renal artery and upper third of the ureter were absent on the right side. The right ureter ended in a small blind bulbous sac in the region of the third lumbar spine. The right adrenal was normal.)*

Total cortex ..... 35.49 gm.

Medulla ..... 10.77 gm.

RATIO 1:3.29

Sample	Weight in grams	Count	Glomeruli per gram
1 .....	0.57	6,000	10,527
2 .....	0.43	4,570	10,628
3 .....	0.49	4,952	10,106

Total weight samples counted ..... 1.49 gm.

Glomeruli in samples ..... 15,522

Glomeruli per gram ..... 10,417

Estimated total glomeruli in kidney ..... 369,700

## DISCUSSION

Comparison of our results with the normal values of Vimtrup<sup>7</sup> demonstrates that the enlarged kidney associated with a congenital anomaly of its mate, contains the usual number of glomeruli characteristic for one kidney of that species. Kidneys 42 and 43 from the same patient, are particularly instructive, since the total in both, 1,322,953 is markedly below the average normal of 1,600,000 to 2,000,000 for man. The number in the enlarged kidney is well within the normal range despite the low total in the hypoplasia.

The count of 1,354,432 in Kidney 108 is slightly above the highest count reported by Vimtrup or secured by us, but the difference is not great and does not invalidate the conclusions.

The ratios of medulla to cortex, of 1:2.87, 1:3 and 1:4.07 in the enlarged human kidneys are greater than normal and indicate a relatively greater increase of cortical tissue than medullary tissue. The reverse is true of the hypoplastic kidney.

The significance of these findings on the function of the congenitally large and small kidney is not, at once, apparent. In all cases, we have noted that there is not an appreciable decrease in the size of the renal artery to the small kidney as compared to a normal

## CASE REPORT

The tumor was found in the urinary bladder of a 4 year-old Herford cow which was slaughtered for food. The animal was in good physical condition, and other abnormalities were not noted on careful postmortem examination. When the bladder was opened, numerous small calculi were found. The mass, which was of a fleshy consistence, was firmly attached to the mucosa of the body of the bladder by a rather broad base. It measured 3 cm. in width at the base and projected into the interior of the bladder approximately 3.5 cm. The growth consisted of one major lobe and two smaller units, separated from each other and the large unit by deep clefts. The surface of the tumor was fairly smooth and possessed many yellowish cystic foci measuring from 0.1 to 0.2 cm. in diameter. The growth did not penetrate the wall of the bladder; it was confined to the interior of the organ.

Microscopically, the growth was made up of a large number of ducts or tubular-like structures, irregular in size and lacking scheme or system in their arrangement, Fig. 1. The ducts consisted of closely packed epithelial cells with a clear cytoplasm and a small granular nucleus situated near the base of the cells, Fig. 2. A definite basement membrane was absent, the cells resting directly on the fibrous tissue constituting the stroma of the growth. The contents of the respective cells of the parenchyma were globular in form and seemed to be of a mucinous nature. Sections stained specifically for mucin gave the characteristic color reaction for this substance. Many large cystic cavities were present, each of which was lined with a single layer of epithelial cells of the same character as those lining the smaller tubules and duct-like structures. The height of the respective cells lining the cysts varied from high columnar to cuboidal; this feature seemed to have a relation to the pressure exerted by the contents of the cysts. The cystic content was of a mucinous nature; apparently it was the product of the cells lining the cavities. Mixed with the mucinous substance of the cysts were enormous numbers of leukocytes with the polymorphonuclear variety predominating. Excretory ducts could not be demonstrated. The stroma was fairly dense in consistence and possessed a few well formed blood vessels, although vascularity was not a prominent

# PAPILLARY ADENOMA OF THE URINARY BLADDER IN THE OX \*

## REPORT OF A CASE

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The occurrence of neoplasms of the urinary bladder in the lower animals apparently is infrequent, if one may draw conclusions from the relatively few cases reported in the literature. In a series of sixty-three malignant tumors affecting the common domesticated animals, Fadyean<sup>1</sup> listed two in twenty-six equine tumors as affecting the bladder. Both of these occurred in mares and were considered to be carcinomas. Cadeac<sup>2</sup> observed carcinoma of the urinary bladder of a horse, with general metastasis to the abdominal organs, and Huynen<sup>3</sup> reported a case of carcinoma of the bladder in a 6 year-old dog. The tumor occupied the anterior extremity of the bladder, and both ureters passed through the mass; the left ureter was open, whereas the lumen of the right was entirely obliterated. Hobday<sup>4</sup> listed papilloma, sarcoma and carcinoma as tumors of the urinary bladder which may be encountered in dogs and cats. He also described briefly a few cases in dogs. Of a total of 1184 carcinomas of the lower animals studied by Sticker,<sup>5</sup> the genito-urinary tract was affected in 195. Primary carcinoma of the bladder occurred in nine dogs, fourteen horses, and nine cattle. Tumors of the urinary bladder were not found by Sticker in cats, sheep or swine. Kitt,<sup>6</sup> in his textbook on comparative general pathology, displayed an illustration of a carcinoma of the bladder of a cow. Fox<sup>7</sup> did not record a case of neoplasm of the bladder in his extensive observations of captive wild mammals and birds.

In my observation of more than 400 tumors in animals, the only tumor of the urinary bladder is the one reported here. This tumor was one of a series of 159 bovine neoplasms, mostly from abattoir cases in which carcasses had been subjected to careful autopsy.

\* Received for publication October 19, 1929.



## SUMMARY

A case of papillary adenoma of the urinary bladder of a cow is described. The tumor was associated with calculi, and a possible etiological relationship is suggested. From available data, it would seem that tumors of the urinary bladder of cattle are not common.

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## DESCRIPTION OF PLATE

## PLATE 43

FIG. 1. An adenomatous portion of the tumor showing its tortuous structure.  $\times 75$ .

FIG. 2. Structural detail of the epithelial cells. A basement membrane is not apparent.  $\times 350$ .

feature. The surface of the growth was somewhat eroded and showed considerable leukocytic infiltration.

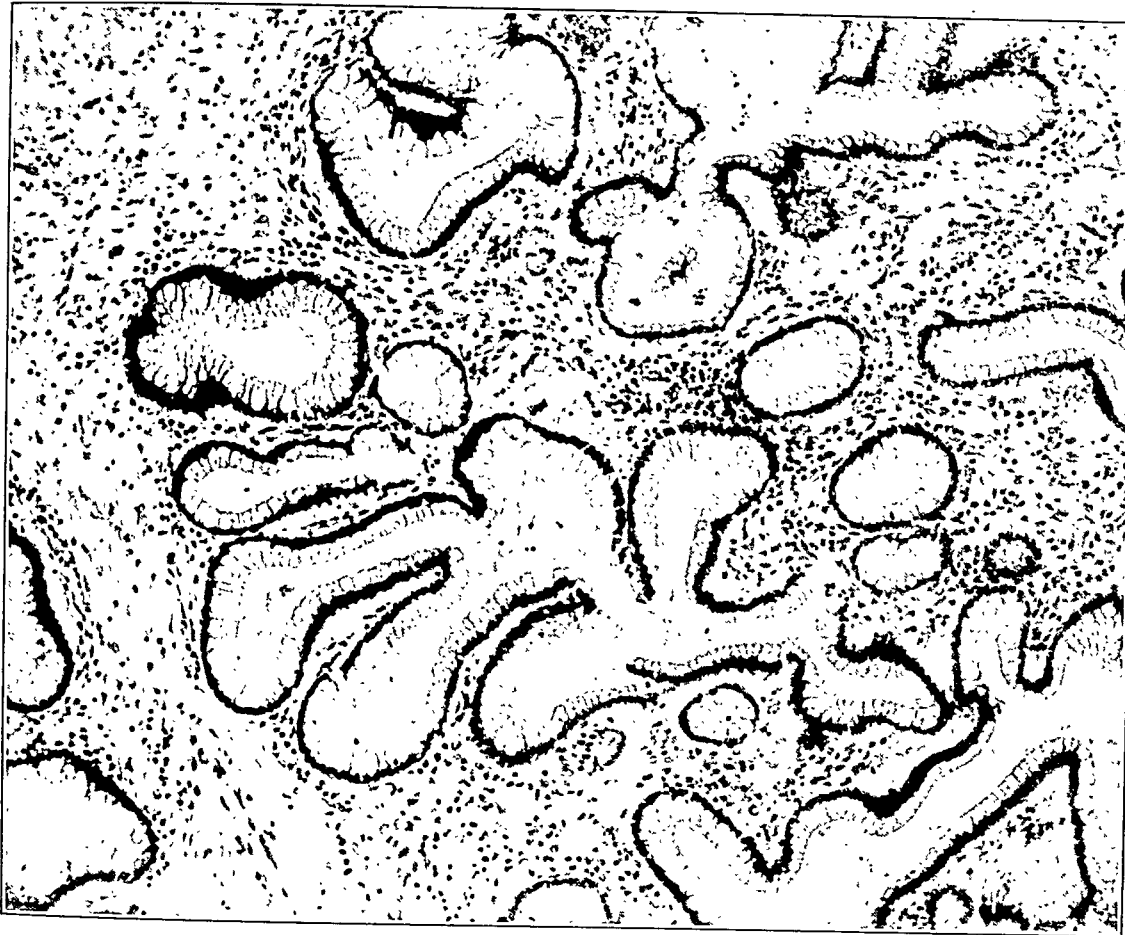
The fact that the growth was of the nature of a polypus without evidence of invasion, together with the failure to demonstrate histologically aggressive, malignant tendencies, would seem sufficient evidence to warrant its classification as a papillary adenoma of the mucoid type.

### COMMENT

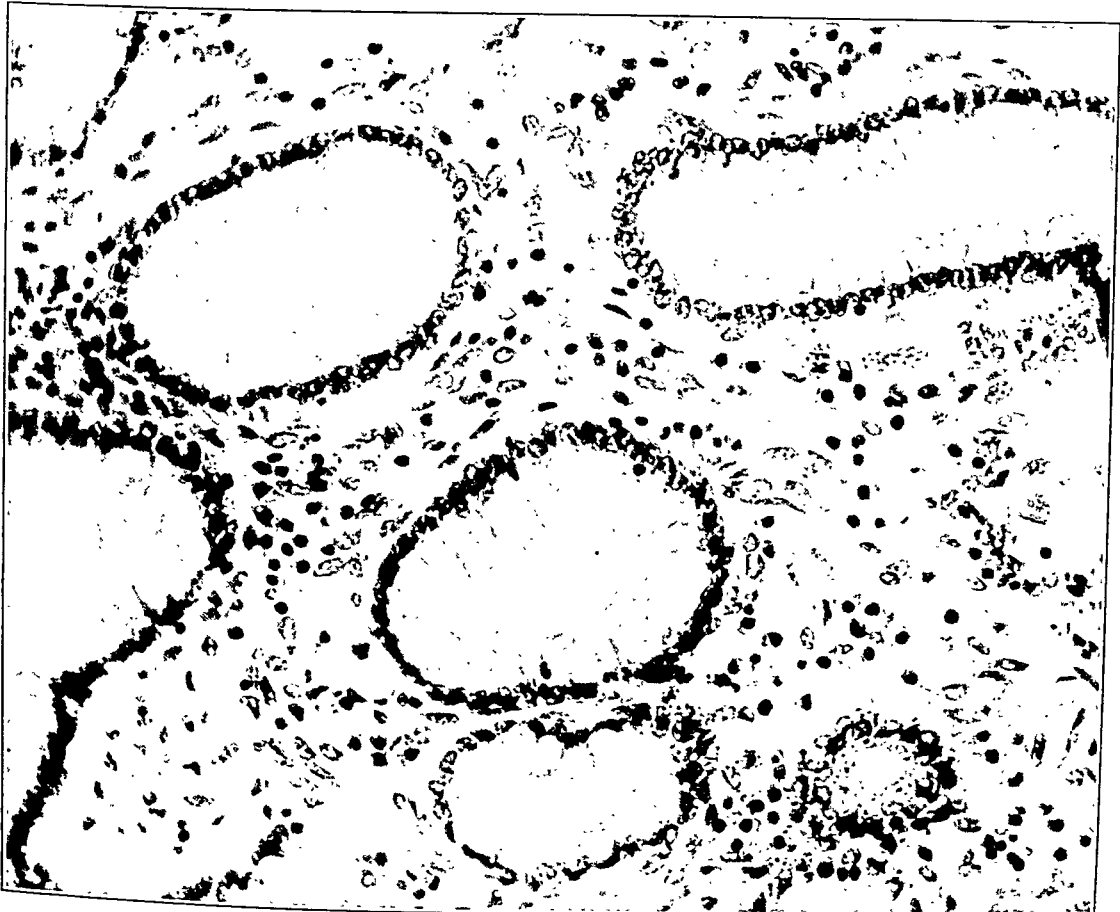
This tumor is of interest because of the comparative rarity of neoplasms affecting the urinary bladder in the bovine species. Although tumors are by no means infrequent in this animal, the urinary bladder usually escapes involvement. The histogenesis of the epithelial cells constituting the parenchyma of this tumor is of some significance. Ewing<sup>8</sup> remarked that in the human being the mucoid type of adenoma of the urinary bladder perhaps has its origin from mucous glands in the epithelial lining of the organ. To determine whether similar glands are present in the urinary bladder of the ox, sections were prepared from several different areas of an apparently normal bladder, and in some sections of the body of the organ well developed mucous glands could be seen. They were not numerous, however, and in sections from some of the blocks none could be demonstrated. Apparently, the histological elements essential to a mucoid type of growth are available in the bladder of the ox.

The presence of calculi in the urinary bladder at once suggests an etiological factor worthy of consideration. Ewing directed attention to the frequency of previous or coexistent cystitis in cases of carcinoma of the bladder of human beings, and the presence of calculi in this case makes it difficult to avoid the thought of a possible relationship between the tumor and the chronic irritation induced by the stones. In this connection it may be noted that Law<sup>9</sup> observed two cases of carcinoma of the bladder in mares "complicated with multiple small calculi and gravel."





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the majority of hearts had a quantity sufficient to cover the proximal portions of the main branches. Towards the apex and the septum the fat diminished in quantity, extending along the ramifications for a short distance. The ascending aorta, which in childhood and adolescence presented slowly increasing delicate streaks of fat running parallel to the aortic-pulmonary groove in front, and upwards from the left coronary behind, in the adult had developed a fair amount of fat along these lines. Small collections of fat extended laterally over the aorta from these. Middle life and old age served to increase all fat bodies abundantly. Frequently the larger coronary divisions became completely embedded.

The fat pads were always most abundant on the anterior surface of the heart. Their extension along the circumflex branches was usually shorter and the amount was smaller. The posterior descending vessels were scantily clad, except in the later decades. Hearts showing marked fat development over the conus arteriosus and the anastomosing vessels of the anterior descending branches often had little fat in the region of the crux behind, or along the posterior coronary branches.

The right heart nearly always showed a preponderance of fat, sometimes this was quite marked. This increase was found most frequently about the right circumflex artery, its anterior descending branches and over the conus arteriosus.

The amount of epicardial and periaortic fat bore no constant relation to the quantity of general body fat. Their extent was related to age and to disease changes in the heart. Their thickness varied. In several obese patients and a few of the undernourished their thickness was increased, in others the reverse was found. Atrophic hearts nearly always possessed a thickened layer of fat regardless of other pathological change.

The fat pads of the dog hearts differed from the human in that those of the ascending aorta were more definitely developed. As described by Davis they formed three distinct collections partially encircling the aorta beneath the pericardial reflection and closely associated with the periadventitial vessels. They were most prominent anteriorly.

The epicardial fat of dog and human hearts was vascularized by innumerable, small, frequently bifurcating branches of the vessels ramifying over the myocardium from the main coronary divisions.

## THE VASCULARIZATION OF THE EPICARDIAL AND PERIAORTIC FAT PADS\*

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Only in recent years have the fat pads of the heart and ascending thoracic aorta been mentioned in the literature. In the human, Gross<sup>1</sup> demonstrated the increase of the arteriae telae adiposae with age, while Campbell<sup>2</sup> noted this increase with both age and cardiac disease. The former thought their development was not entirely secondary to functional need, and that they were an expression of a general body nutritional reserve. He described a heart in which the fat pad vessels had prevented infarction in the region supplied by the almost completely occluded ramus circumflex dexter. The latter illustrated their function by a case of infarction in which an occluded coronary artery was so bridged by arteriae telae adiposae that infarction did not occur in the area involved. Davis<sup>3</sup> described the periaortic fat bodies of the dog, and studied them in various other animals and the human. These masses increased in size with age, and in their development bore no relation to the amount of general body fat. Woodruff<sup>4</sup> found the fat pads of the dog very vascular and well developed. They were frequently present immediately over one of the discrete vasal openings in the ascending thoracic aorta.

To further the above observations, the coronary and ascending aortic vessels of dog and human hearts were injected with various solutions. The vascularization of their fat bodies was then studied in the gross and microscopically by serial sections and preparations cleared in oil of wintergreen.

In the human, the fat pads were found accompanying the coronary vessels of all adult hearts. They were seen in the infant as small collections of fat covering the coronary vessels before they divided, or extending over the divisions for a short distance. In childhood and adolescence these collections of adipose tissue increased along the sulci occupied by the circumflex, marginal, and anterior descending divisions of the coronaries. In early adult life

\* Received for publication October 26, 1929.

mosis between the thoracic and the coronary vessels was established, best developed over the conus arteriosus.

A variation was noted in the number of vessels in the fat pads. Those hearts with slight or moderate pathological change and abundant fat pads showed relatively fewer arteriae telae adiposae than hearts involved in fibrosis and coronary sclerosis.

Two injected human hearts showing fibrosis and other lesions illustrated the above general observations on fat pad development and its vascularization.

CASE 1. The first case (Autopsy 56-29) a male aged 72 years, died of recurrent erysipelas of one month's duration. He had typhoid fever thirty years previously, but gave no sign of arteriosclerosis or of heart disease during life. The heart showed abundant fat pads, much more marked than usual over the ascending aorta, pulmonary artery and the conus arteriosus. There was marked sclerosis of both coronary arteries. The first inch of the left coronary was completely calcified and its first branch was given off one inch from the sinus of Valsalva. The right coronary showed a small branch just at its mouth, but three small accessory and one large accessory coronary artery were present, through which a rich anastomosis was formed in the fat pads over the conus arteriosus with the left anterior descending coronary branches, and over the ascending aorta with the pericardial vessels and the vasa of the arch. The fat pad vessels and those of the ascending aortic limb were much increased in size.

CASE 2. The second heart was from a male, aged 73 years (Autopsy 58-29). He claimed he had never been ill until eight months before death when he suffered shortness of breath, and for the last two months swelling of the feet and legs. Preceding death for several weeks there was increasing edema extending to the chest, and marked dyspnoea. The heart was much hypertrophied, the fat pads were increased over the pulmonary artery, the ascending aorta, the conus arteriosus and over the anterior surface of the heart. The left ventricle towards the apex showed a large thrombus over a very thin and ballooned fibrotic area of myocardial infarction. The left coronary was very sclerotic and almost occluded in its first portion, giving rise to only one small auricular vessel to the fat pads of this side. The second branch of the left coronary, running between the pulmonary artery and the aorta to the right side of the aortic wall, was very small. The right coronary branches were also very small

These branches spread beneath the epicardium and anastomosed with similar ones from the same vessel or from neighboring vessels. They also spread deeply toward and into the muscle, where they ended by ramifying and anastomosing with the muscular branches of the coronaries which penetrate the myocardium perpendicularly to the surface. A very freely anastomosing vascular network was thus formed between the neighboring coronary vessels.

When the epicardial fat, extending along the branches of the right and left coronary divisions, met, as over the conus arteriosus in many human adults, there was a similar network and anastomosis between their vessels.

The fat bodies of the ascending thoracic aorta were vascularized in the above fashion by the periadventitial vessels which they surrounded. In the human, these periadventitial vessels usually arose as right or left coronary branches. In adults, those descending from the pericardium, the arch of the aorta, or the bronchial arteries were occasionally covered by fat which they vascularized. In the dog, small vessels from the lumen of the ascending aorta joined the periadventitial network. A profuse anastomosis was thus present over the ascending limb even in young dogs, and the fat bodies were correspondingly increased in dimension.

Human epicardial fat was scarce or microscopic in amount at birth and often abundant at old age. This increase and the degree of right and left coronary anastomoses had a definite correlation. Hearts well supplied with epicardial fat presented a dense network of vessels ramifying over the heart and sending branches into the myocardium. A widespread and rich anastomosis between various coronary vessels was then established.

The development of the ascending aortic fat bodies was significant. With age the periadventitial vessels proliferated, enlarged and spread over the aortic wall, while the fat surrounding them also increased and grew along their course. Frequently the aorta was almost completely encircled by collections of fat which covered the ascending limb from the pericardial reflection to the heart. In such cases, periadventitial branches of the coronaries to the ascending aortic limb, together with similar branches descending from the arch of the aorta, the pericardial and the bronchial arteries, along with the innumerable fat pad vessels, formed a richly anastomosing bed of vessels over the aortic wall. Through this network a free anasto-



In eighteen autopsies on adult cases of obesity, the epicardial fat was increased only in four by an amount corresponding to that of the general body fat. Eight hearts showed a moderate increase in epicardial fat, while six showed no increase. But with the exception of one, these hearts were moderately or severely damaged by chronic inflammatory change. The increase of epicardial fat corresponded closely to the extent of myocardial and coronary injury.

In a series of fifty-four adult cases where chronic interstitial myocarditis and other cardiac lesions were found at autopsy, three were obese. Fifty-one had only a moderate or a decreased amount of general body fat, as observed in the subcutaneous tissues, abdominal wall and omentum. Thirteen of these showed an abundant increase in epicardial fat; eleven a moderate increase. Twenty-three had the usual amount present in adults, four less epicardial fat than usually seen. Thus, in twenty-four of these hearts, epicardial fat was more abundant than in the average adult heart. In all it was greatly increased over that found in childhood and adolescence. Thus, although the fat bodies increase roughly with age, their development depends upon the reaction of the myocardium to an unhealthy environment and to disease processes which are initiated in youth and progress with years.

In the two cases reported, coronary and myocardial damage had apparently been marked for many years. The increase in number and size of the periaortic and epicardial arteriae telae adiposae, and of the ascending aortic periadventitial vessels formed a widespread anastomotic network which supplied the myocardium.

### SUMMARY

1. The arteriae telae adiposae of heart and ascending aorta proliferate in response to disease, augmenting the myocardial blood supply or tending to compensate any deficiency in it.

2. The periadventitial vessels of the ascending aorta, joining the coronary vessels with those of the thorax, may greatly assist in this compensation.

3. The fat bodies develop about the proliferating vessels and their size depends primarily on the extent of vascularization present, secondarily on such factors as atrophic and sclerotic myocardial

and sclerosed, but several ran over the ascending aorta for a short distance. However, the vessels from the arch and the pericardium were markedly increased in size and number, and spread over the ascending aorta into the fat pads about the right coronary artery and over the conus arteriosus, to establish an anastomosis with branches of the left coronary divisions.

## DISCUSSION

The work of Gross, Campbell, and Davis, and that presented here, demonstrates that the fat pads increase with age and disease, and are not dependent in their growth on general body fat development. The fat pads are always best developed and appear first over the anterior aspect of the ascending aorta and heart where anastomoses are readily demonstrated and reach their greatest development. They increase and extend with the spread of the ascending aortic and coronary anastomoses. The dog with the peculiar vascularization of the ascending aorta possesses well defined aortic fat bodies. The fat pads always covered or surrounded the coronary or periaortic vessels which gave them an abundant and widely anastomosing blood supply.

The right heart usually shows a preponderance of the epicardial fat. This may depend on the relatively greater activity of the left heart over the right, on the predisposition of the right heart to atrophic changes and on the reaction of the myocardium to disease. The left heart, more prone to arteriosclerotic changes in its vessels, relies to some extent on the right heart for the establishment of a compensatory blood supply. The anastomoses appear largely between the right and left anterior descending branches and over the conus arteriosus, both being closely related to the thoracic anastomoses over the ascending thoracic aorta. When the inflammatory response to infection has passed, the newly formed vascular bed, more abundant over the right heart, becomes surrounded by fat, replacing atrophied and sclerosed tissue.

The variations in the amount of the fatty deposit in relation to the number of arteriae telae adiposae, and the increased deposit of fat in atrophic hearts, would indicate that the thickness of the fat pads depended to some extent on cardiac inflammation and on peculiarities in metabolism.

## DESCRIPTION OF PLATES

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### PLATE 44

FIG. 1. Distribution of adult fat pads.

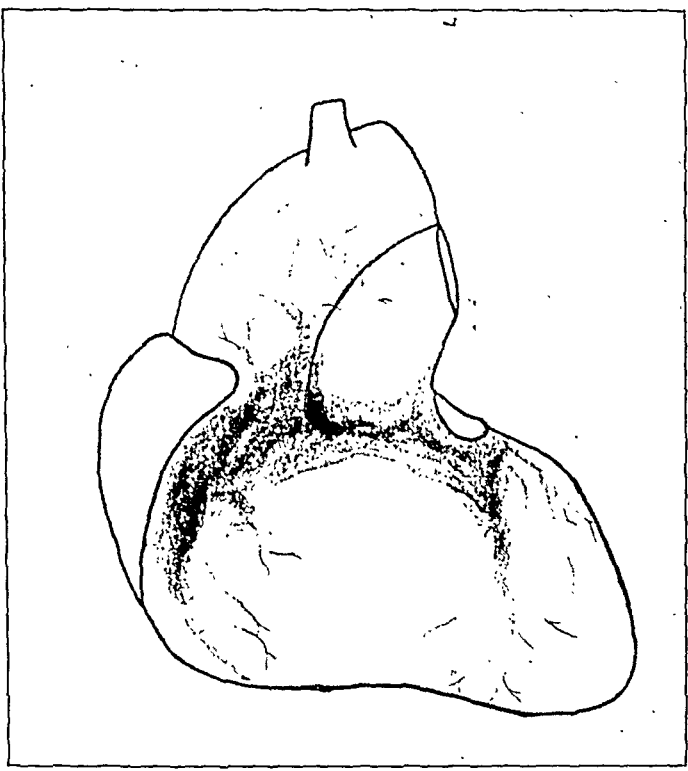
FIG. 2. Distribution of arteriae telae adiposae and ascending aortic periadventitial vessels.

changes, and individual peculiarities in local and general metabolism.

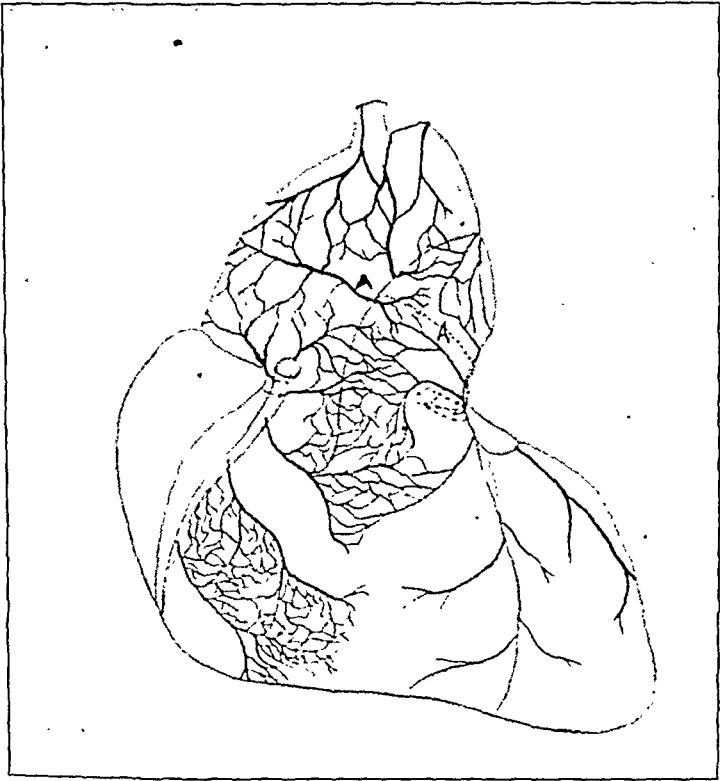
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acquired the idea, little by little, of a submucous muscular mechanism, then of a submucous musculonervous complex, present in all appendices. My brief description in 1924 and again in 1928 has attracted no attention. If I now return to the subject it is because this complex seems to me worthy of notice by its constancy and by its peculiarities, both in the normal and the pathological state.

As we shall see, marked variations in the musculonervous complex are habitually accompanied by obvious changes, always of the same order, in the other appendicular constituents, always involving the submucosa, often the mucosa and sometimes the muscular coat. From this observation arose the idea that these changes are not unconnected with those of the mechanism itself and that their study is capable of throwing light on its significance. I shall not, therefore, limit the description to the complex itself but, in connection with each example studied, I shall indicate the condition of the other appendicular structures.

## I. APPENDICES OF THE NEW-BORN

*Normal Type:* (Figs. 1 and 3.) In the first place we must describe this complex in the surely normal appendix, that of the new-born or of the nursling. At this age the appendicular mucosa is habitually poor in lymph nodules and rich in crypts of Lieberkühn. The muscularis mucosae forms an almost continuous layer beneath the tips of the crypts, interrupted only in the region of certain lymph nodules.

The submucosa is thin and already fibrous. Study of the bundles of circular muscle surrounding it shows that from time to time certain bundles change their course and pass obliquely into the fibrous connective tissue in which they seem to lose themselves. In the submucosa, centrally from these oblique muscle bundles, we find other, more slender bundles presenting at most eight to ten contractile fibers abreast, which seem to be isolated in the fibrous connective tissue. From the muscularis mucosae in the same sector, muscle bundles here and there detach themselves and bury themselves in the submucosa. Serial sections show that the muscle bundles of each group, the one issuing from the circular muscle, the other from the muscularis mucosae, converge and anastomose in the middle of the submucosa. Sometimes one or two muscle bundles cross this middle zone and join the two groups in one network.

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## CONTRIBUTION TO THE STUDY OF THE SYMPATHETIC NERVES OF THE APPENDIX. THE MUSCULONERVOUS COMPLEX OF THE SUBMUCOSA\*

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Describing the lesions following acute appendicitis, pathologists speak of cicatricial scleroses of the submucosa which invade the circular muscle and dissociate its bundles more or less. Their illustrations show the muscle bundles separated from one another and turned from their normal course so that some of them seem to lose themselves in the fibrous connective tissue of the submucosa; the boundary between the submucosa and the circular muscle is no longer so clearly marked as in the normal intestine.

Beyond question, up to a certain point, postinflammatory sclerosis explains this partial dislocation of the muscularis and the musculoconnective tissue interpenetration; but, if this were the only cause, we should be obliged to admit that all appendices without exception have been diseased, even those of nurslings and of the new-born, for all of them present in different degrees the same ill-defined boundary between the circular muscle and the submucosa. From this observation what conclusion can be drawn other than that this lack of precision of the boundary does not result exclusively from sclerosis, but that it corresponds at least in part to a normal structure? This was the point of departure of the present study.

I first observed this condition in beginning my investigation of nerve proliferation in the appendicular mucosa and the origin of argentaffin cell tumors (carcinoids). While pursuing this work I

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instances of chronic appendicitis, the lymph nodules are continuous or even heaped up in several layers, leaving space for only a small number of crypts beneath which no trace of a muscularis mucosae can be found. In these specimens a few bundles are seen to detach themselves from the circular muscle, but no contractile (smooth muscle) cell continues through the lymphoid tissue, encroaching on the submucosa. The internal half of the mechanism is wanting, as is the muscularis mucosae itself.

In children and adolescents, after one or more appendicular crises, quite frequently there is seen a reversal of the conditions just described, consisting in rarefaction of the lymph nodules and sclerous thickening of the submucosa. Often in this thickened submucosa the muscular mechanisms are more prominent than in the normal state. These variations indicate already on the one hand an inverse proportion between the richness of the mucosa in lymph nodules and in the number of the crypts; on the other hand a certain correlation between the gland content of the mucosa and the development of the muscular mechanisms of the submucosa. These contacts and relations are much more obvious in the adult, as we shall see.

### III. ADULT APPENDICES

All pathologists know how variable is the number of lymph nodules in the adult appendix. Study of a large number of specimens shows that on the average the number of these nodules is less than in children and that at times they are almost or completely absent. This lack of lymphoid nodules is habitually associated with former appendicular symptoms, acute crises or chronic appendicitis without acute inflammatory attacks, as if the spontaneous lymphoid involution had been hastened by these inflammations.

1. *Appendices with Numerous Lymph Nodules, Normal Type:* (Fig. 4.) The aspect of the mucosa is similar to that observed in the adolescent. The muscular mechanism presents no notable change. It is always found in the sectors provided with crypts.

In one specimen, exceptional it is true, I observed a mechanism more typical than usual (Fig. 2). Instead of showing along its whole length a plexiform structure, it was reduced to a perfectly straight and radial bundle visible in its entire length in one section, extending between the muscularis mucosae and the circular muscle,

I cannot say that this union is constant. The oblique course of the bundles makes it difficult to draw a conclusion. However, union is beyond question in certain instances where each group is conical in form. The base of one is continuous with the circular muscle, that of the other with the muscularis mucosae. Their apices are joined by one or two more or less oblique anastomotic bundles. In other specimens the ordinary location of the two groups in the same sector and the direction of their bundles toward each other indicates a certain correlation between them, even if they are not actually joined together. They form a whole which I propose to call the muscular mechanism or complex of the submucosa.

Each cross-section of an appendix shows elements belonging to several (two to four) of these mechanisms. We should note also these two points: (1) The arteries destined for the submucosa travel among the bundles emanating from the muscularis mucosae; and (2) Meissnerian nerves are always present between the bundles of a whole complex. As to their relations with the muscular mechanism, the evidence is not so good as that which we shall be able to offer later on.

## II. APPENDICES OF CHILDREN AND ADOLESCENTS

*Normal Type:* Appendices removed from children and adolescents are much richer in lymph nodules, although their number and size vary greatly, not only from one specimen to another but also in different regions of the same specimen. Sometimes they are isolated, sometimes grouped in masses and more or less continuous at their borders. They may be small and lodged entirely in the mucosa, or they may encroach on the submucosa; and in this event, the most frequent, there is no muscularis mucosae beneath them.

Crypts of Lieberkühn are found only in the spaces between the lymph nodules at points where the nodules are not too closely approximated. It is only at these points, remaining glandular, that the muscularis mucosae persists. It is in these sectors provided with a muscularis mucosae, and consequently with crypts of Lieberkühn, that we find the anastomotic muscular mechanisms with the features already described. The submucosa is two or three times thicker than at an earlier age.

*Pathological Types:* In certain subjects in whom lymphatic tissue is developed to the utmost, such as status lymphaticus and certain

muscles and nerves of the submucosa. This may be the only lesion but it is often accompanied by a curious alteration in the nerve plexus of the mucosa, to which may be added hypertrophy of the muscle coat and even of Auerbach's plexus. In this order, then, we shall study them: first the submucosa, followed by the mucosa and the muscularis.

### SUBMUCOSA

The thickness of the submucosa, always excessive, varies from 0.25 to 0.5 cm. It is constantly fibrous, poor in fat cells but always of loose texture, especially in the middle. In this region there is often an imperfect cleavage into two concentric layers, the one fusing with the mucosa, the other with the circular muscle.

The muscular mechanisms and Meissner's plexus are prominent, obviously hypertrophied. The hypertrophy may affect only Meissner's plexus; its nerves are broad, elongated and tortuous, the ganglia larger, more numerous and richer in cells than in the normal state while the muscular mechanism, moderately but distinctly enlarged, contains only a few bundles. In the neighborhood of the circular muscle these bundles are thick; toward the muscularis mucosae they are slender and plexiform, always mingled with Meissnerian nerve filaments.

The Meissnerian hyperplasia, though present, may be masked more or less by hyperplasia of the muscular mechanisms, the contractile fibers striking the attention by their bright color (Fig. 8). The muscle bundles continuous with those of the circular muscle occupy the outer half of the submucosa. They ramify in different directions. Some of them converge toward the middle zone of the submucosa forming cones broader and better furnished with fibers than in the normal mechanisms; others run parallel with the axis of the appendix and seem to establish longitudinal anastomoses between the hyperplastic mechanisms from one segment of the appendix to another. Whatever direction they take the bundles are cylindrical. In the neighborhood of the circular muscle their fibers run parallel with one another. As they bury themselves in the submucosa the fibers become oblique, wavy, and they arrange themselves in the interior of the bundle in fascicles anastomosing with one another so that each bundle has a plexiform structure.

The nerves, hyperplastic and studded with tiny ganglia, ramify

to which latter it was attached by two or three oblique bundles. A branch of Meissner's plexus wound around it like a climbing plant. An artery of the mucosa traversed the muscularis mucosae in the immediate vicinity of its insertion.

2. *Appendices with Scanty Lymph Nodules, though Present in all Complete Cross-Sections. Passage from the Normal to the Pathological Type:* Sometimes the muscular mechanisms resemble those described above: they correspond to the normal type. Sometimes their bundles are obviously larger and more numerous. The mechanisms themselves are not increased in number. Their general form remains the same but they are seen more readily because of the greater number of their constituent bundles.

Along their whole length these mechanisms are mingled intimately with Meissnerian nerve filaments, distinctly larger and more numerous than in the normal state. These filaments, studded with tiny ganglia, form a plexus between the muscle bundles.

3. *Appendices with Very Scanty or Absent Lymph Nodules. Pathological Types:* (Figs. 5, 6 and 7.) This extreme diminution of lymph nodules is seen especially in the adult, seldom in the adolescent. Such an appendix almost invariably has a clinical history; repeated crises or a syndrome of chronic appendicitis. Its external appearance varies greatly. The length and caliber may be normal. It may be short, 3 to 4 cm., and correspondingly broad, 1 to 2 cm., or the tip may be swollen in the form of a pendulum. More rarely it is enlarged in all dimensions, 10 or even 15 cm. long, 1 to 2 cm. broad. Often it is buried in fibro-adipose peritoneum.

On examining sections with the naked eye, after fixation, the most striking features are the extreme narrowing of the lumen to such a degree that it seems to have been obliterated, and the thickening, often enormous, of the appendicular wall. The thickening involves the submucosa especially, but also in addition in extreme examples, the muscularis.

Appendices which combine these three fundamental features, narrowing of the lumen, extreme diminution or absence of the lymph nodules and thickening of the submucosa, present also more or less complex changes in their histological constituents. At first sight their situation, nature and extent seem to be capricious. Study of a large number of specimens has enabled me to establish a certain order among them. The most constant change affects the

With these musclonervous hyperplasias are constantly associated important changes in the arteries. In the outer zone of the submucosa the lumina of the vessels are enlarged; their walls are thickened by hypertrophy of the muscle fibers as well as by interstitial sclerosis. Often there is notable thickening of the intima. The adventitia presents a nerve plexus of large tortuous fibers. The branches of the arteries directed toward the mucosa are more numerous than normal, tortuous, winding and mingled with muscle and nerve fibers. In short, all of the nervous and muscular elements of the submucosa undergo more or less hyperplasia, those of the arteries as well as those which belong to the musclonervous complexes.

### MUCOSA

Except for its poverty in lymph nodules the mucosa of the appendix may present nothing abnormal (Fig. 8). The crypts of Lieberkühn are numerous, straight and parallel as in the colon. Their tips may extend to the immediate vicinity of the muscularis mucosae. The mucosa does not contain an excessive number of nerves and the meshes of its stroma are filled with lymphocytes, plasma cells, and usually a few polymorphonuclear eosinophiles.

However, it may present more or less pronounced hyperplasia and even neuromas of the subglandular portion of the intramucous plexus. In this event the nerves always contain argentaffin cells, the form and origin of which I have described elsewhere. The meshes of the subglandular stroma, formed in large part by the hypertrophied nerves, are reduced to narrow clefts filled with lymphocytes. These cells are less numerous as the nerve hyperplasia is more pronounced. There results a relatively clear appearance of the deep region of the mucosa, which I have emphasized in former writings as a probable sign of hyperplasia of the subglandular plexus. Nerve lesions of this kind have no relation to the musclonervous hyperplasia of the submucosa. They may coexist, but the fact that the nerve lesions may be found without submucous hyperplasia shows that they do not depend upon it, in contrast to those which we are about to describe.

Here too the mucosa presents a stroma clear and poor in lymphocytes; but this appearance, instead of being restricted to the subglandular region and due to hypertrophy of its nerve fibers, extends

among the muscle bundles, often run along with them and give off branches which encircle them or penetrate their interior to follow a course parallel to the fascicles.

The inner half of the submucosa presents many muscle fibers, sometimes grouped in bundles intimately anastomosing, sometimes interlaced in a close-meshed plexus. Anastomosing with those of the muscularis mucosae these fibers may group themselves in plexiform sheets that parallel the muscularis mucosae and reduplicate it externally. These fibers or these bundles converge toward the summits of the muscle cones emanating from the circular muscle and anastomose with them here and there.

The interstices of this robust muscle plexus are occupied by a very rich and delicate nervous plexus containing many ganglion cells, and by arteries destined for the mucosa (Fig. 15). The abundance of the nerves, and especially of the muscles, varies within wide limits. In certain specimens their mass may be estimated at one-half to two-thirds of the tissue present in the submucosa; that is to say, the total thickening of the submucosa is due in great part to their presence.

Such is the aspect of the musculonervous complex in most examples of lymphoid atrophy of the mucosa. However, I shall now describe the curious structure presented in some of my specimens by those muscle bundles, which issuing from the circular muscle assume a longitudinal direction (Figs. 16 and 17). These large bundles, round in cross-section and plexiform in structure, are not in direct contact with the connective tissue of the submucosa but are separated from it by a continuous sheath of varying thickness formed by a plexus of non-medullated nerve fibers. The nerve fibers are voluminous and appear edematous. The nerves sheathe the bundles only in their longitudinal ramifications; when the bundles take another direction the nerve sheath resolves itself into ordinary filaments. The nerve fibers are pressed closely together, anastomosing in a plexus of meshes elongated in the direction of the muscle bundles which they surround. Here and there the sheaths present small sympathetic ganglia with large cells. From time to time they are seen to give off small branches which penetrate the bundles and disappear between the muscle cells. This peculiar structure, though rare, shows very well the remarkable union of muscle and nerve in the complexes which are the subject of this study.

tinuous with the reticulum of the adjacent mucosa. These neuromas never contain ganglion or argentaffin cells.

(b) The second and, in my experience, the most frequent form is situated between the crypts, stretching out parallel to them in the upper three-fourths of the mucosa (Figs. 12, 13, and 14). Some of these tumors are swollen in pendulum form, the pedicle occupying the level of the tips of the crypts while the swollen portion often reaches the superficial epithelium between two glandular orifices. These neuromas may be numerous, twenty-five to thirty per cross-section. Their structure is identical with that of the rounded neuromas just described. Over their entire surface they are continuous with the nerve plexus in the mucosa. In certain specimens their pedicle is in direct continuity with nerves emanating from the hyperplastic plexus of Meissner.

In short, whether diffuse or spherical, elongated or pendulum-shaped, these neuromas appear to be the result of localized hyperplasia of the nerve plexus of the mucosa, a hyperplasia characterized by lengthening of the fibers and the formation of spheres. The fibers remain very slender and are enclosed in relatively thick hyaline collagen sheaths. These neuromas are continuous with the hyperplastic plexus of Meissner of the submucosa. They never contain argentaffin or ganglion cells. Contrary to the argentaffin cell neuromas, which are situated exclusively between the muscularis mucosae and the tips of the crypts, they occupy the interglandular region of the mucosa and extend as far as the basal epithelium lining the lumen of the appendix.\*

#### MUSCULARIS AND AUERBACH'S PLEXUS

Other changes, inconstant but, when they exist, always in combination affect: (1) The muscular coats, especially the circular muscle. The bundles are large and formed of hypertrophied fibers similar to those of the gravid uterus (Fig. 8). This hypertrophy involves even the smooth muscle of the meso-appendix. (2) Auerbach's

\* I should say here that all attempts at silver impregnation of these hyperplastic nerves, those of the mucosa as well as those of the musclonervous complexes, have been futile. Critics will doubtless say, as has already been charged concerning the argentaffin cell neuromas, that I have seen nothing but neurinomatous proliferation. I must reply that, while positive impregnation of neurites has great value, a negative result proves nothing either way, considering the inconstancy of the method, especially for the myenteric sympathetic of the appendix.

through the entire thickness of the mucosa. In addition, the stroma seems to be edematous and the enlarged meshes of its reticulum contain abundant fluid poor in free cells.

Often this edematous mucosa is thickened. The crypts are sometimes straight, sometimes sinuous and broadened. In this event their epithelium, as well as that of the appendicular lumen, presents some anomalies, larger, taller and broader cells clearly hypertrophied and hollowed by deeper calices than normal. Argentaffin cells are few (Figs. 12 and 14).

In studying this edematous mucosa as colored with the trichrome stain, an extreme richness of nerves is often observed. These nerves form a large part of the meshes of the reticulum but, in contrast to that which occurs in argentaffin cell nerve hyperplasia they are remarkably slender, containing one, two or at most three neuroglia tubes abreast. In certain specimens the nerves form multiple neuromas, which we shall now consider.

1. In the deeper half of the mucosa, there are diffuse neuromas which occupy large sectors. Their fibers are slender and closely approximated, leaving no room between them for a lymphatic space. Their course is capricious. Often they run more or less parallel and form whorls. These neuromas surround the lower half or lower third of the crypts. Their borders are ill-defined and continuous with the more loosely-meshed plexus of the general mucosa.

2. Circumscribed neuromas, always numerous and of small dimensions, which occupy the middle or the middle and upper region of the mucosa.

- (a) The first group (Figs. 9, 10 and 11), consists of small round masses which after staining with the anilin blue trichrome appear as small, round blue spots situated between the tips of contiguous crypts, never between them and the muscularis mucosae. Their diameter averages one and one-half times that of the crypt. Their number may be enormous: I have counted forty in each cross-section of an appendix. They consist of a spherical mass of non-medullated fibers pressed closely together and anastomosing, united to the rest of the plexus of the mucosa by many fibers escaping from its surface. Each fiber of the neuroma is enclosed in a relatively thick collagen sheath, hyaline and fused together, giving the whole mass its blue tint with the trichrome stain. The collagen sheath is permeated by a network of argyrophil fibers (Laidlaw's technique), which are con-



carried to the extreme. At my request, Professor Oberndorfer had the kindness to send me some of his preparations. I recognized all of the features which he has described and illustrated so perfectly and my impression is that his giant appendix fits into the category of lesions described in this paper: thickening of the mucosa, lengthening of the crypts with hypertrophy of the cells, enormous diffuse intramucous neuroma, submucosa thickened and containing a gigantic nerve plexus and many muscle bundles, hypertrophy of Auerbach's plexus and thickening of the muscle coat.

At first sight, some details seem to differ from those that I have observed. Thus, in his Figures 37 and 38, Oberndorfer (1929) represents the "nerve" bundles surrounded by ganglion cells. I question if these bundles are really nervous and not muscular, enclosed in a nerve sheath like those in my illustrations. I also question if the "syncytiale Bänder" of his Figure 39 do not relate to muscle rather than to nerve elements or, more exactly, neurinomatous elements. Oberndorfer's stains, (hematoxylin and eosin, and iron hematoxylin) do not permit a definite answer to these questions.

Finally, Oberndorfer notes thickening of the nerves of the meso-appendix. I have observed nothing of this in my specimens, but none of them offers such extraordinary nerve hyperplasia as that of Oberndorfer. Is it not plausible that when pushed to the extreme the nerve hyperplasia may extend beyond the parietal plexus of the appendix and include the nerves of the meso-appendix itself?

In view of the participation of its various constituents in the enlargement of the appendix, Pick concludes that gigantism is "co-ordinated with" a fault in embryonic development, an excessive and tumoral growth of the local nervous system. For him, as for Oberndorfer, it is a congenital malformation. This view may be valid in certain instances. In my opinion it cannot be applied wholly to those which form the subject of this paper nor, perhaps, to Oberndorfer's specimen. While the influence of an excessive development of the sympathetic nerves on the growth of the other constituents of the appendix appears to me to be plausible enough, there is nothing in the instances cited in this paper to prove that the excessive growth has an embryonic origin. Its constant occurrence after frank crises, its primary situation in Meissner's plexus of the submucosa, its extension to a mucosa impoverished in lymph nodules

plexus itself. The nerves are more voluminous and the ganglion cells are two or three times larger than normal: the filaments ramifying among the muscle bundles are prominent and studded with numerous ganglia.

When an appendix presents all of these hypertrophies and hyperplasias at once, muscular, nervous, vascular and even epithelial, its dimensions may become considerable; its length may attain 12 to 15 cm. and its external diameter 3 cm. Most frequently the length remains normal and the diameter only is increased.

In sections stained by ordinary methods, the most striking features are the size and abundance of the nerves and ganglion cells. In the submucosa the abundant elongated nuclei may be mistaken for nuclei of Remak, whereas a portion of them, usually the larger portion, belongs in reality to the muscle fibers, as shown by the trichrome stain.

### DISCUSSION

The lesions just described are of frequent occurrence. Not a week passes that my collection is not enriched with two or three specimens of musculonervous hyperplasia of the appendicular submucosa. Only the extreme examples with intramucous neuromas, hypertrophy of Auerbach's plexus and of the muscle coats and gigantism are relatively rare. Study of a large number of specimens shows that these giant appendices do not form an isolated group but that they are connected with the normal appendix by an infinite number of intermediate types. That these latter have not been described hitherto is doubtless due to the universal employment of techniques (hematoxylin and eosin, and Van Gieson), which do not reveal the muscles and nerves of the mucosa with sufficient clearness to strike the attention.

The literature contains a few observations, some of which should be revised in the light of the facts related here. Various authors have described ganglioneuromas, neurofibromatosis, Rankenneuromas, ganglioneuromas with or without gigantism of a given portion of the intestine. The site of the lesions is sometimes the small intestine (Pick, Baltisberger), sometimes the appendix (Heine, Oberndorfer, Schmincke, Schultz). The descriptions of these last four authors, especially that of Oberndorfer, suggest the thought that their specimens might represent musculonervous hyperplasia

the intestinal epithelium, just as the existence of nerves is bound up with the presence of the ganglion cells on which they depend. If, then, the hypothesis of the neuron is applicable to these neuromas it is certain that we must seek their trophic centers in the argentaffin cells and not elsewhere.

These observations have led me to the idea of a nervous system peculiar to the intestinal mucosa, of entodermic origin, a neurentoderm, characterized by the presence of ganglionic, neuroglial and secretory cells provided with silver-reducing granules, the neurites of which, indistinguishable by Cajal's methods, mingle with those of the sympathetic and form the plexus of the mucosa. This plexus then would contain two kinds of fibers, the one, centripetal, belonging to the neurentoderm, the other, centrifugal, starting from the sympathetic ganglia and arriving at the intestinal surface. Because of the organogenic analogies of this hypothetical system with the argentaffin cells and the placodic neuro-epithelium of the olfactory mucosa, I have thought that its function might be sensory rather than motor.

However, the conditions described in this paper have a totally different aspect. Meissner's plexus undergoes hyperplasia, either of its fibers only or of its ganglion cells also. There is a corresponding hyperplasia of the muscular mechanisms and of the arteries of the submucosa. At a further stage the hyperplasia involves certain nerve filaments of the mucosa, the centrifugal fibers just pictured which remain slender, lengthen and form superficial neuromas without argentaffin cells. At a still more advanced stage the muscle coat and Auerbach's plexus hypertrophy in their turn. These appendices have no tendency whatever to obliteration. If it occurs it is not the result of regression of the epithelium but of inflammatory ulceration. It is obvious that the site of these hyperplasias is a motor nervous system and the muscles which depend on it, above all the Meissnerian level of the intestinal sympathetic nervous system and the muscular mechanisms of the submucosa.

The neuro-argentaffin hyperplasias on the one hand and the sympathicomuscular hyperplasias on the other may exist in a pure state, which demonstrates their reciprocal independence and the independence of the two systems of nerves from which they arise. They are not incompatible. They may coexist in the same ap-

and enriched in crypts, then to the muscular coat and, finally, its frequency, seem rather to make of it an acquired lesion. This excessive growth is probably the result of appendicular inflammations, associated on the one hand with nerve regeneration following ulceration and on the other hand with a new growth of the mucous plexus more or less perfectly adapted to the new crypts that have replaced the involuted lymph nodules.

Other studies on the appendix have enabled me to verify the existence of nerve hyperplasias different from those described here, situated in the subglandular portion of the mucous plexus and there only. They are preceded by the migration of cells from the intestinal epithelium into the nerves. After penetrating the nerves these cells become loaded with silver-reducing granules and differentiate in various ways, some taking the form of ganglion cells, others of cells of Remak, while still others, cylindrical or spherical, elaborate fats which they eliminate directly into the nerves. Subsequently these nerves grow, lengthen and broaden to the point of forming neuromas; they are always situated beneath the tips of the crypts and are capable of pushing the muscularis mucosae into the submucosa (Fig. 18).

The epithelial covering of the mucosa often disappears and the lumen of the appendix becomes obliterated. The neuromas survive and even increase in obliterated appendices; they disappear if their argentaffin cells disappear. Their origin and their persistence are thus bound to the presence of these cells just as the existence of nerves of any kind is bound up with the presence of cells.

The impossibility of demonstrating neurites in these "neuromas" by the usual methods of silver impregnation has been urged as an objection to my views, and most writers have concluded that these growths are really neurinomas. In accepting this latter view we should be obliged to admit that the greater part of the nerves of the normal appendicular mucosa do not contain neurites either, because silver impregnations are negative for them also. Is this not placing too great reliance on the most capricious, the least reliable of techniques?

From another viewpoint the proliferation of Schwann cells in authentic neurinomas exhibits remarkable autonomy, but, in the argentaffin cell neuromas, proliferation and persistence of the nerve filaments is controlled by the presence of the cells emigrated from

nodules, scarcity of lymphocytes in the stroma, and diffuse hyperplasia of its nerve plexus, to which may be added more localized hyperplasias in the form of ill-defined neuromas or circumscribed neuromas situated in the middle or upper part of the mucosa. In extreme examples there is in addition hypertrophy or hyperplasia of the muscle coat and of Auerbach's plexus.

To sum up, there is hyperplasia and hypertrophy either of the sympathetic nerves or of the appendicular muscles, or of both, in other words, of the motor apparatus of the appendix, together with atrophy of the lymphoid tissue. This series of lesions seems to begin by the formation of these musculonervous complexes.

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pendix, either in different regions or associated in the same sector, each preserving its own characteristics (Fig. 19).

Why not, then, admit the existence in the appendix of two groups of nerves, associated, mingled, indistinguishable in the normal state but recognizable under pathological conditions by their isolated or combined hyperplasias? The one is the argentaffin group, the other the myenteric group. Have these two groups distinct origins, as I am inclined to believe, or are they both of sympathetic origin, as stated by most neurologists? New researches will doubtless answer this question.

The significance of the musculonervous complex remains for me quite obscure. It is not easy to understand what mechanical rôle could be played by these contractile bundles buried in the submucosa. Nor is it easy to picture the organogenic processes of which they might be vestiges. They belong exclusively to the appendix. Does not their close association with motor nerves permit us to compare them with the neuromuscular nodes described by Keith in the muscularis of the colon, so little studied since, which play a part in intestinal motility? Still another problem.

### SUMMARY

The submucosa of the normal appendix contains muscle bundles, continuous on the one hand with those of the circular muscle and on the other hand with those of the muscularis mucosae. These two muscle groups often anastomose in the middle zone of the submucosa. They are in intimate relation with certain parts of Meissner's plexus. Muscle and nerve together form a complex which we may term the musculonervous complex of the appendicular submucosa.

Under influences that we cannot yet define, but connected no doubt with inflammatory crises, the muscle bundles and the nerves become more numerous, acquire more and more intimate relations, and by their accumulation contribute to the thickening of the submucosa. At the same time the arteries enlarge, their muscle coat hypertrophies and the arterial nerves become more prominent.

The mucosa presents certain changes which in the order of frequency are: decrease in number or complete absence of lymph

## DESCRIPTION OF PLATES

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### PLATE 45

FIG. 1. Appendix removed from an infant of 4 months during an operation for congenital hernia. General view of a sector of the wall. Cross-section. (*m*) mucosa; (*mm*) muscularis mucosae perforated in the center by an arteriole destined for the mucosa and interrupted at the edge of the photograph by a primitive follicle; (*sm*) submucosa; (*c*) circular muscle; (*l*) longitudinal muscle; (*p*) peritoneum.

Note the muscle bundles (gray) anastomosing with those of the circular muscle, which invade the submucosa where they form a plexus continuous with slender muscle bundles emanating from the muscularis mucosae (cut nearly transversely) near the point of penetration of the artery. In the photograph the bundles appear as small gray circles near the broad lymph capillaries. This group of bundles forms a muscle complex. With the magnification employed, the nerves of Meissner's plexus ramifying in their meshes are not visible. This appendix is represented in Fig. 3 in its entirety.

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PLATE 46

FIG. 2. Normal appendix from a patient 20 years of age. Above, the deep region of the mucosa rich in lymphoid tissue and bordered by the muscularis mucosae. Below, the circular muscle. Between the two, the submucosa traversed by a muscle bundle which unites the circular muscle and the muscularis mucosae. A Meissnerian nerve (black) accompanies it and winds around it. In this section it is visible only in the external portion of its course, but in serial sections it may be followed as far as the neighborhood of the muscularis mucosae where it ceases to be impregnated by the silver.

This musculonervous complex is typical in its simplicity but infrequent. A complete section of this appendix is represented in Fig. 4.



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PLATE 47

All the appendices represented in this Plate have been photographed with the same magnification in order to show the differences between normal appendices and those affected by hyperplasia of the musclonervous complex of the submucosa.

FIG. 3. Infant of 4 months (see Fig. 1). Broad lumen, mucosa rich in crypts and poor in lymphoid follicles, submucosa thin, muscle coat thin.

FIG. 4. Male, 20 years of age (see Fig. 2). Lumen relatively narrow, mucosa rich in lymphoid tissue, submucosa thicker, muscle coat scarcely thicker than in the infant.

FIGS. 5, 6 and 7. Different degrees of hyperplasia of the musclonervous complex of the submucosa. Lumen narrow, mucosa rich in crypts, poor in follicles (6) or deprived of follicles (5 and 7). Muscularis mucosae continuous and thickened. Enormous thickening of the submucosa which splits incompletely into two concentric layers. Thickening of the muscle coat.

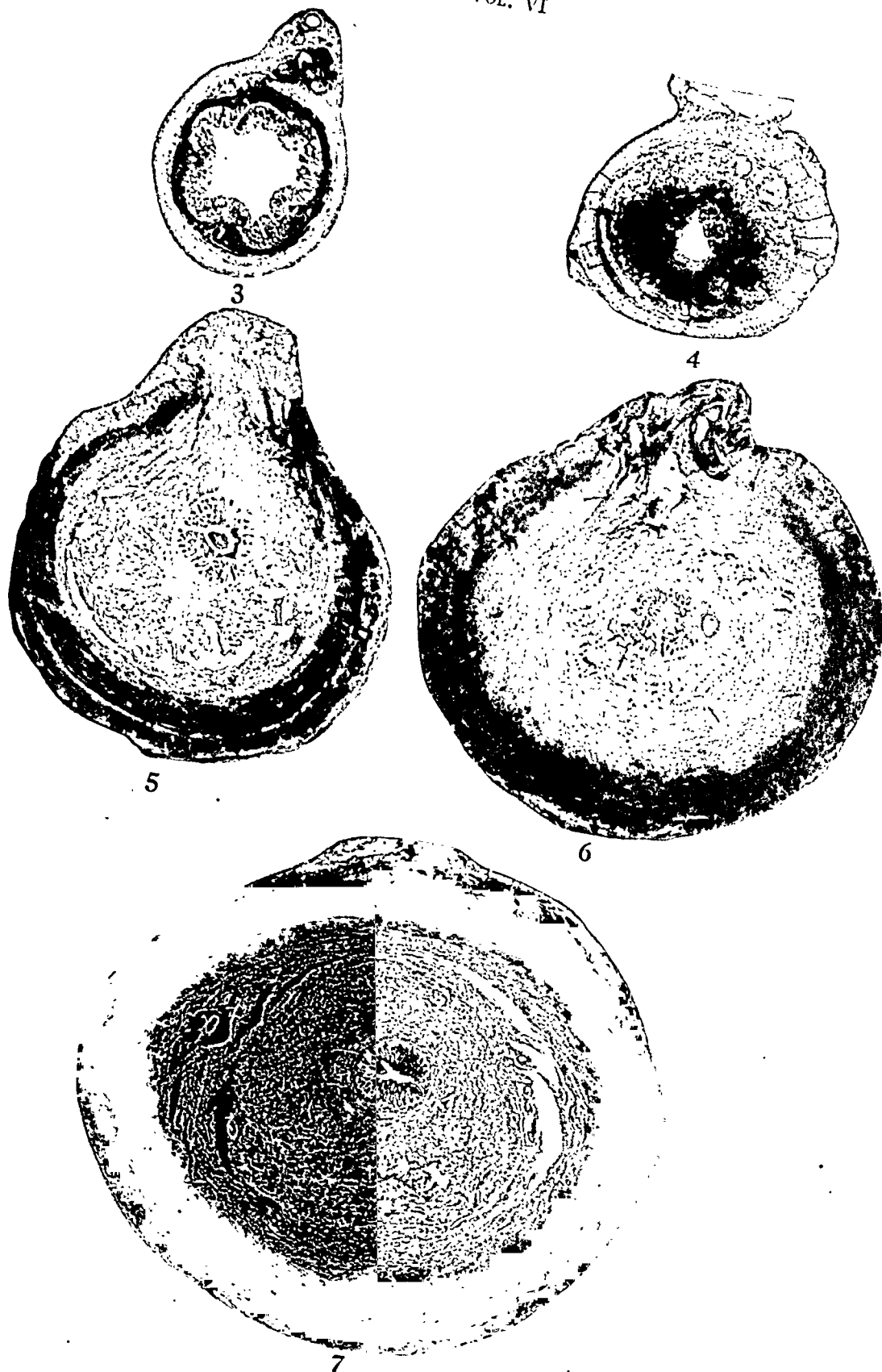


PLATE 48

FIG. 8. Appendix of Fig. 6 enlarged. (Obs. 126 M.) Trichrome.

Mucosa rich in crypts, the stroma clear, edematous, poor in lymphocytes. There are no intramucous neuromas in this specimen. Muscularis mucosae thickened. Submucosa enormous, crowded with muscle bundles in the interstices of which the connective tissue (black) is scanty. In the middle zone of this submucosa may be seen arterioles with hypertrophied walls.

Circular muscle greatly hypertrophied. No interstitial sclerosis. Its limits on the side of the submucosa are indistinct on account of the many muscle bundles that escape to form the hyperplastic muscle complex of the submucosa. Longitudinal muscle coat scarcely thickened. Peritoneum thin.



Masson

Musculonervous Complex of Appendix

PLATE 49

FIG. 9. (Obs. 142 M.) Trichrome.

General view of a mucosa containing many spherical neuromas. Thirty may be counted in the figure. One of them is represented at a higher magnification in the corner of the figure.





PLATE 50

FIGS. 10 and 11. (Obs. 142 M.) Laidlaw's silver technique.

The reticulin which surrounds the nerve fibers is continuous with that of the stroma of the mucosa: it leaves the fibers colorless while outlining the contours clearly.

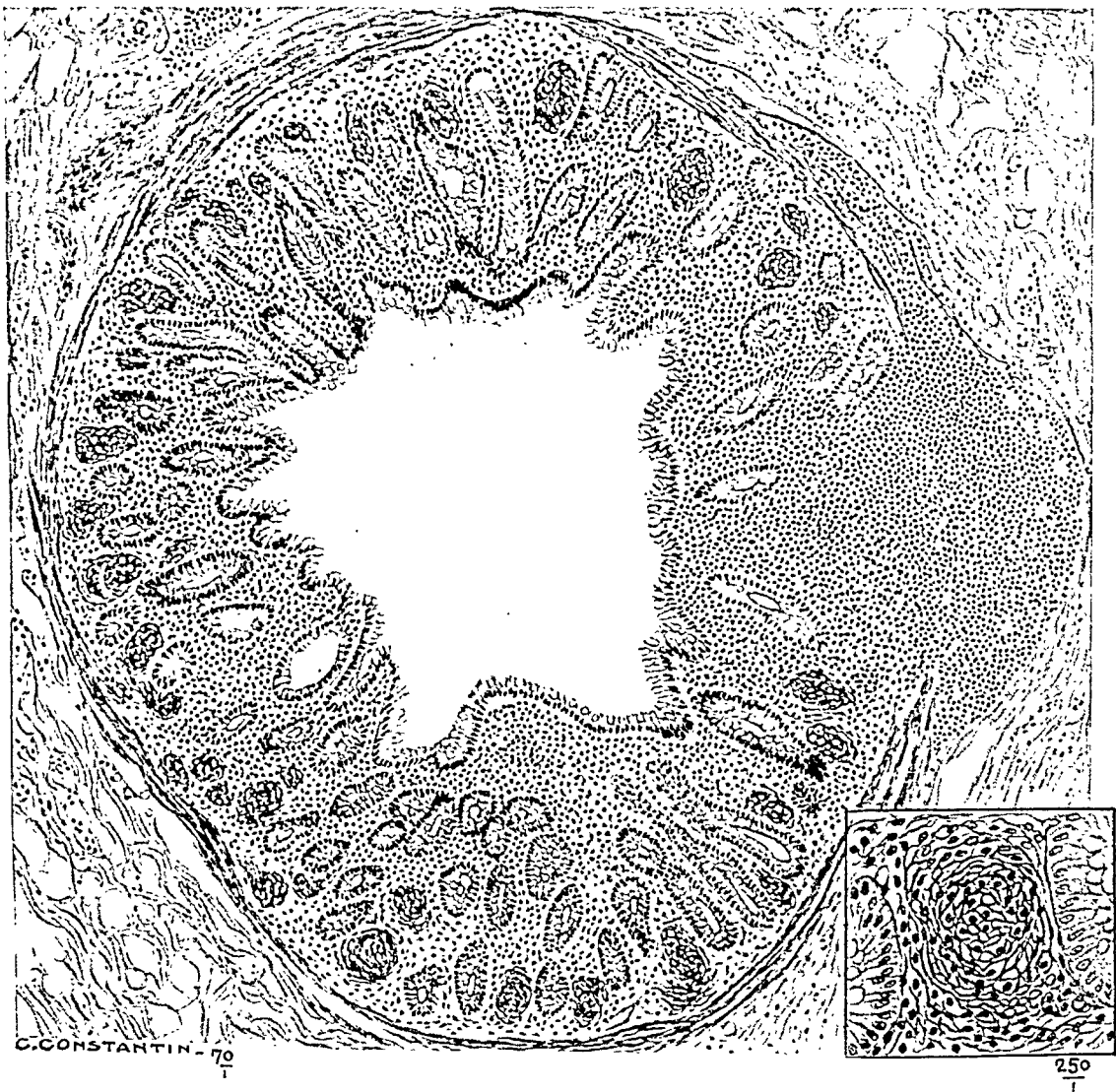


PLATE 51

FIG. 12. Same case as that of the general view, Fig. 7. (Obs. 68 M.) Trichrome.

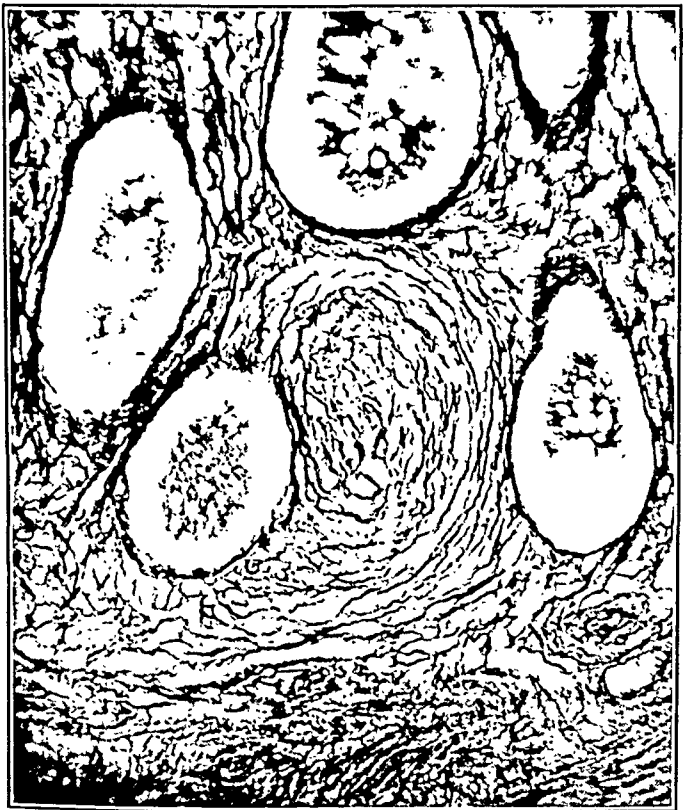
Intramucous neuromas in pendulum form. Mucosa thickened, edematous. Crypts large and tortuous. Between them are two elongated neuromas connected by their bases with a diffuse neuroma which dissociates the muscularis mucosae and continues as a very broad Meissnerian nerve. The submucosa is traversed by innumerable nerve filaments invisible with this magnification.

FIG. 13. (Obs. 68 M.) Laidlaw's silver technique.

Two neuromas in pendulum form, their summits reaching the basal epithelium of the appendix. Here again only the contours of the nerve fibers are outlined by the reticulin which is continuous with that of the stroma.



10



11

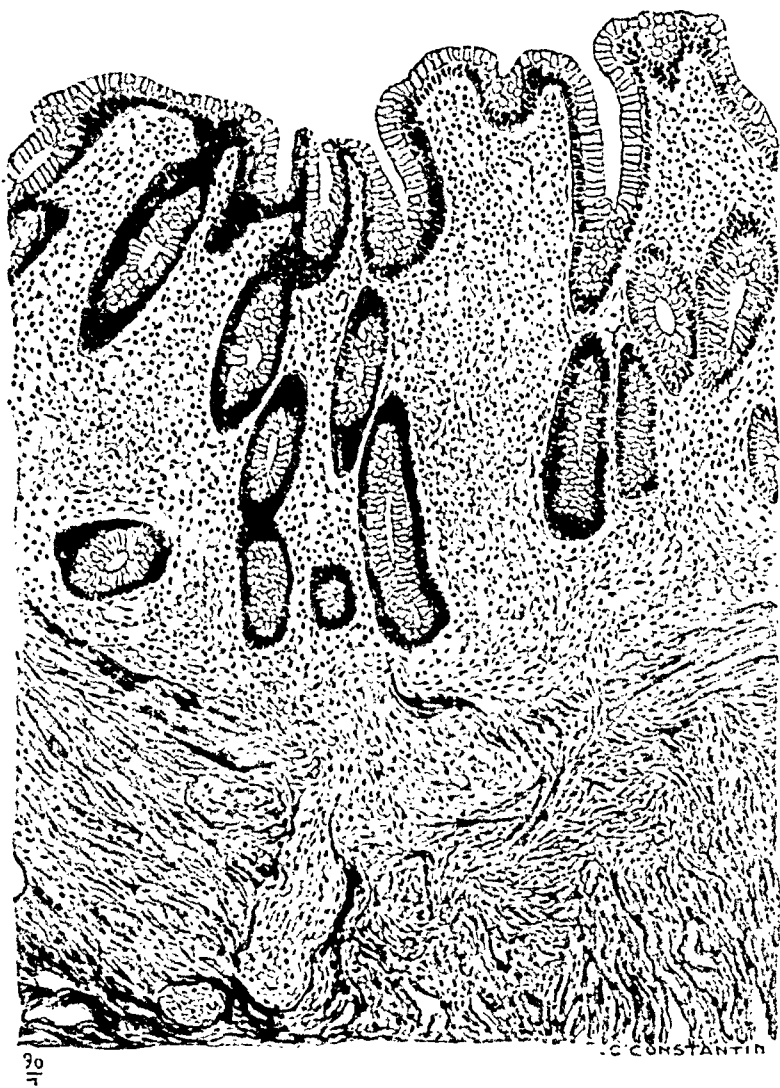
PLATE 52

FIG. 14. (Obs. 68 M.) Trichrome.

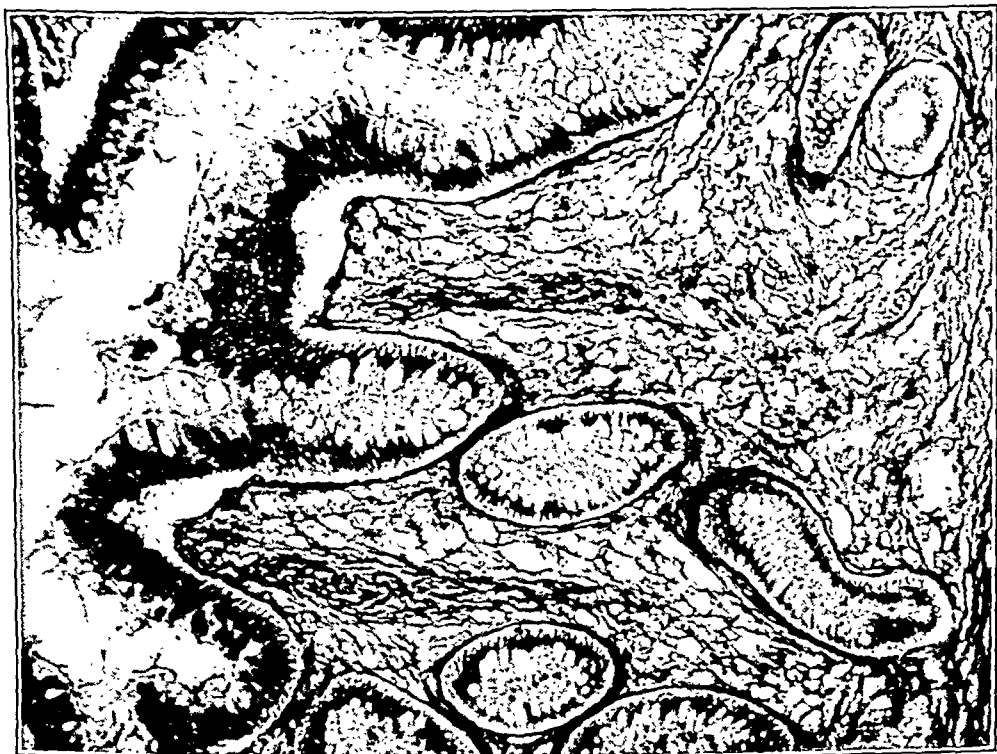
Section of the mucosa near the surface and almost parallel to it. Note the considerable hypertrophy of the epithelial cells. Between them is a neuroma cut through the swollen portion. Note its irregular form, its compact texture, the thickness of the collagen sheath around each nerve fiber, the relative pallor of the nuclei of Remak and the continuity of these fibers with those which form an important part of the reticulated framework of the adjoining stroma.

FIG. 15. (Obs. 126 M.) Trichrome.

Hyperplasia of the muscilonervous complex of the submucosa. Submucosa in the neighborhood of the muscularis mucosae. Confused interweaving of non-medullated nerve fibers recognizable by their spongy aspect, and muscle fibers oriented in every direction and sketching here and there small bundles. Fine nerve fibers are seen in the interstices between the muscle cells. A ganglion cell.



12



13

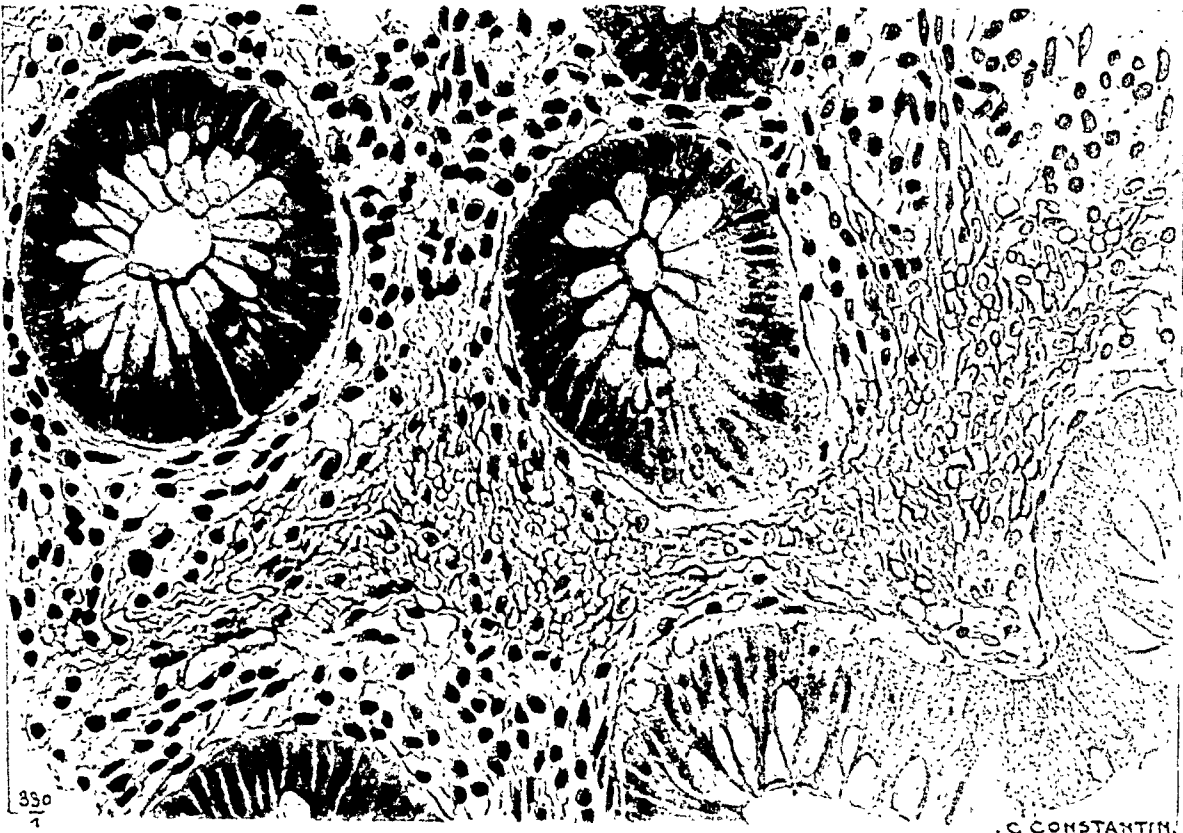
PLATE 53

FIG. 16. (Obs. 126 M.) Trichrome.

Submucosa in the vicinity of the circular muscle. A muscle bundle in a longitudinal direction in the neighborhood of a bifurcation. This bundle is surrounded by a continuous nerve sheath of longitudinal fibers. A large ganglion cell in the nerve sheath.

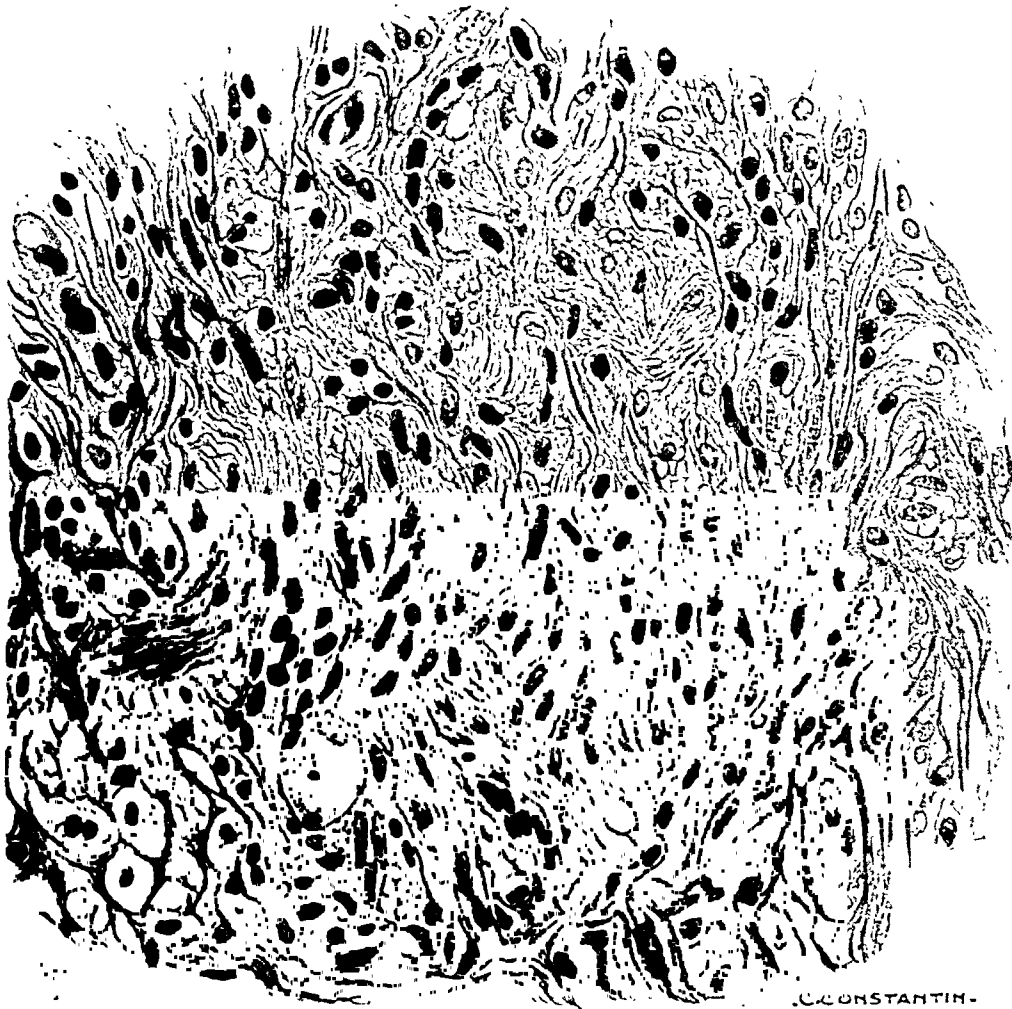
FIG. 17. (Obs. 126 M.) Trichrome.

The same musculonervous bundle after its bifurcation. Each branch has its own nerve sheath. A small sympathetic ganglion in the nerve sheath.



C. CONSTANTIN.

14



C. CONSTANTIN.

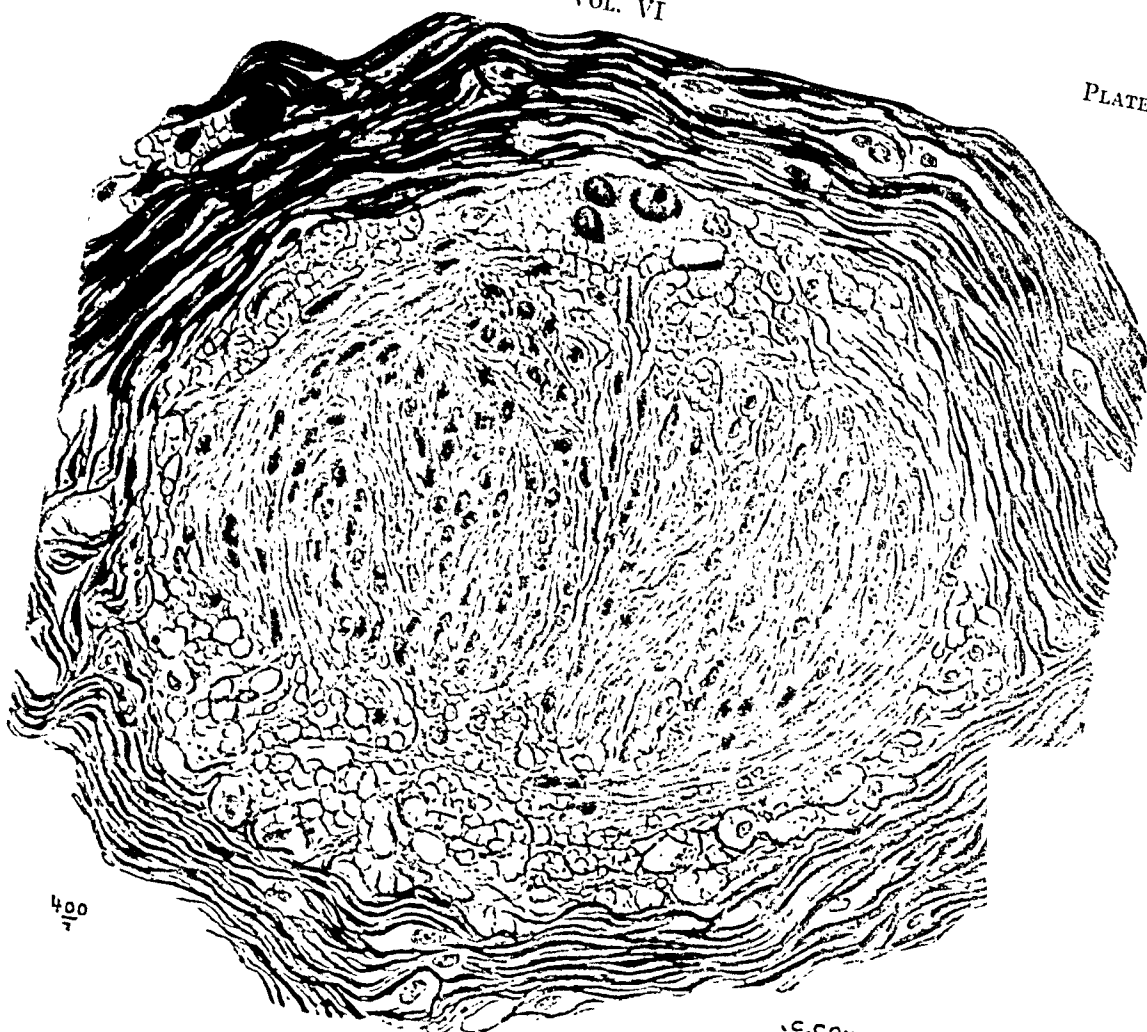
15



PLATE 54

FIG. 18. (Obs. 118 M.) Trichrome.

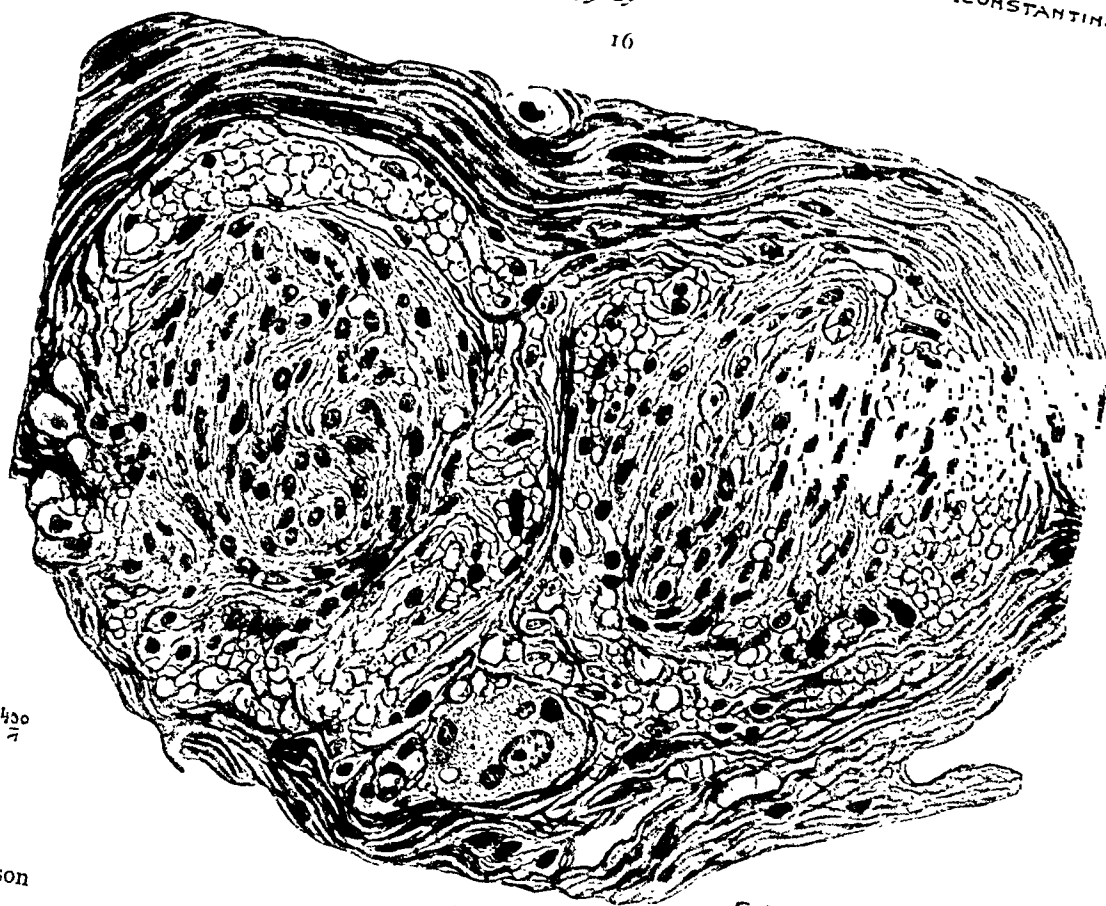
Intramucous argentaffin cell neuroma forming a clear mass between the tips of the crypts and the muscularis mucosae which is crowded into the submucosa.



400  
7

.C.CONSTANTIN-

16



400  
7

.C.CONSTANTIN.

17

Masson

Musculonervous Complex of Appendix

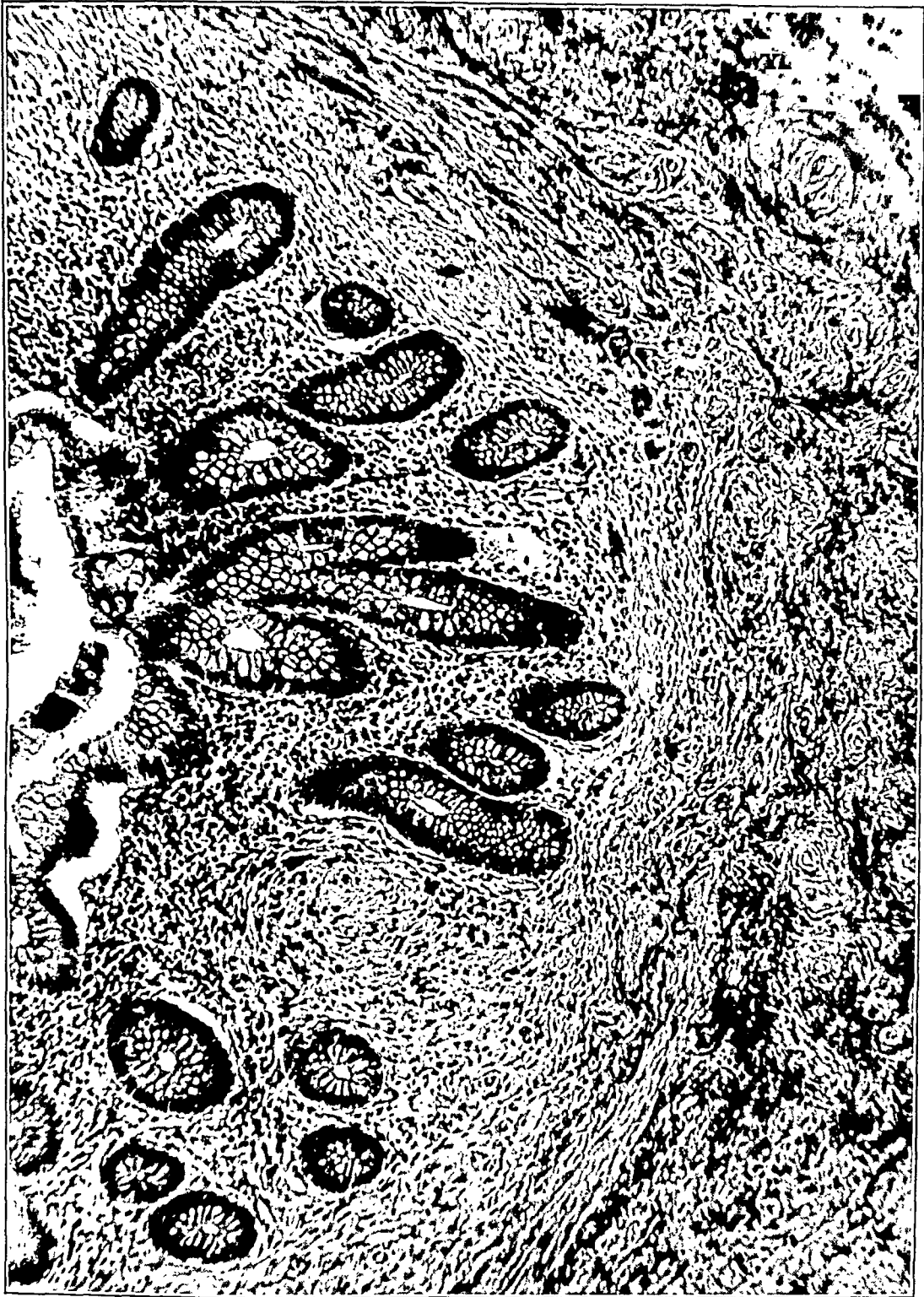
PLATE 55

FIG. 19. (Obs. 126 M.) Trichrome.

Association in the same region of the mucosa of a small, argentaffin cell neuroma (above), developed between the tips of the crypts and the bulging muscularis mucosae, and a pendulum-shaped neuroma connected with hyperplasia of the musclonervous complex of the submucosa (below).







As regards her present illness, the classical acute symptoms of gall bladder disease started four days before admission with colicky pains starting in the epigastrium, radiating along the right costal margin to the middle of the back, then to the right shoulder and arm, occasionally to the right thigh. This lasted one to three hours, leaving a residual skin tenderness in the right upper quadrant of the abdomen. Her urine became dark brown and the stools clay-colored. The jaundice had been present for the past six months. This attack was accompanied by chills and fever. The patient complained mostly of the vomiting, which was usually preceded by pain in the nape of the neck. This vomiting was food qualitative — strawberries, meats and tea. Fat, however, was tolerated well. No blood was seen in the vomitus which was occasionally green.

Physical examination showed a thin, under-nourished, slightly jaundiced female rambling from one point to another in giving her history, which was occasionally interrupted by threatened vomiting attacks. Clavicles were prominent. The expiratory phase at the apices was prolonged. Inspiratory râles were present posteriorly at the angles of the scapulae. Apices showed increased tactile fremitus posteriorly. Tenderness and spasm were found in the right upper quadrant of the abdomen. The knee jerks were equal. No clonus, Babinski, Kernig or Romberg signs.

Roentgen examination showed no gall stones. The duodenal cap was normal. The barium passed out of the stomach rapidly, massively filling the duodenum. Reverse peristalsis was noted in the duodenum. There was a slight six-hour gastric retention, most of the barium being in the terminal ileum, the ascending and first portions of the transverse colon.

The preoperative urinalysis was negative for sugar, albumin, acetone and diacetic acid. Kahn and Wassermann tests on the spinal fluid were negative.

Twenty-four hours after operation the patient suddenly developed an acute respiratory paralysis. She responded to artificial respiration but stopped breathing again after a few minutes. Further artificial respirations for at least two hours were of no avail.

Autopsy performed one hour after death gave the following unexpected findings: Multiple small angiomas of the cerebral cortex, multiple dural meningiomas, a pressure cone of the cerebellum and medulla, terminal bronchopneumonia, bilateral healed apical tuberculosis of the lungs, caseous tuberculosis of the right pulmonary lymph nodes, emphysema of the lungs, chronic adhesive pleuritis on the right side, atheroma of the aorta, mild nephrosclerosis, and a recent laparotomy wound for cholecystectomy.

#### GROSS FINDINGS IN THE BRAIN

The dura was stretched tense over the brain. The spinal fluid was clear. Through the external surface of the dura over the right frontal, parietal and temporal regions many opacities of various sizes could be seen, which later were found to be due to the presence of multiple meningiomas adherent to the inner dural surface. The

# MULTIPLE INTRACRANIAL ANGIOMAS \*

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## INTRODUCTION

When one considers that multiple intracranial angiomas are very rare, the case reported here is most unique in that it was associated with the equally rare multiple intracranial meningiomas. Lindau<sup>1</sup> and more recently Dandy,<sup>2</sup> have made detailed studies of the venous abnormalities and angiomas of the brain, with excellent reviews of the literature on the subject. The general consensus is that these tumors are the result of a congenital anomaly of the vascular system of the brain.

## REPORT OF CASE

*Clinical History:* M. H., white, female, married, 34 years of age, was admitted to the service of Dr. John A. Sampson, Albany Hospital, for cholecystectomy and freeing of numerous dense adhesions around the gall bladder.

As regards her past history the patient had had the usual diseases of childhood, appendectomy in 1914, pneumonia, and dilatation and curettage in 1919. She was pregnant three times, the last two being miscarriages which were followed by severe hemorrhages. Four years ago the patient had the usual picture of gall bladder disease with jaundice and clay-colored stools but refused operation. At that time she also complained of nocturnal (two or three times), and diurnal frequency of urination of moderate amounts accompanied by burning. Her urine, however, was negative. Blood sugar was 97 mg. per 100 cc., and basal metabolism minus 10 per cent. Other complaints were shortness of breath on exertion, palpitation of the heart and swelling of the ankles. Physical examination of the cardiovascular system at that time did not reveal anything remarkable. She had no cough, expectoration, night sweats or chest pains, but she gave a history of having had pulmonary tuberculosis several years before.

The patient had had no domestic difficulties, fits of anger or hysterical spells, but she had been having occasional dizzy spells and a feeling of pressure in the head. For the past three years she had been suffering from periods of depression lasting one to two days. During these periods she would cry a great deal. Because of these depressive states she was advised to enter the Poughkeepsie State Institution for observation but she refused to sign voluntary papers of commitment.

\* Received for publication December 26, 1929.



In the various sections of the cerebral cortex all the cortical vessels stand out prominently, due to a marked hyaline and fibrotic thickening of the capillary wall (Fig. 4), and to the widely distended perivascular Virchow-Robin spaces. The nearer one gets to the angiomatous tumor areas the more prominent and the more numerous these vessels become. Numerous capillary angiomas of microscopic size are found scattered throughout the cortex of the frontal lobe. The smaller tumor areas consist of a complicated, branching, anastomosing network of capillary vessels, so strikingly shown in frozen sections stained by Penfield's method (Fig. 5). Very little collagen and no elastic fibrils are demonstrable in the younger angiomatous areas as shown by the Van Gieson, Weigert and Verhoeff stains. The older tumor nodules consist of closely packed hyaline masses in the center of each of which is a capillary lumen, either empty or containing red blood cells. Collagen is abundant and coarse elastic fibrils are demonstrable here and there in some of these older tumors. Reticulum is present only in the wall of the finer capillaries; none is found where there is increased collagen deposition.

In the white matter, just outside the distended perivascular spaces, there is a dense accumulation of numerous hyaline, spherical "amyloid" bodies. In those sections taken from the various parts of the brain, microglia and oligodendroglia are not increased in size or number.

### DISCUSSION

Compared with the other intracranial newgrowths, angiomas are not frequently observed. No mention of an angioma is made among 258 verified intracranial tumors by Tooth,<sup>5</sup> among 42 verified tumors by Heuer and Dandy,<sup>6</sup> 40 verified tumors by Greenfield,<sup>7</sup> or among 117 verified tumors by Purves-Stewart.<sup>8</sup> Cushing<sup>9</sup> found 8 or 0.9 per cent angiomas among 868 verified intracranial tumors. In a review of 100 verified intracranial growths, Dowman and Smith<sup>10</sup> found one angioma or 1 per cent. However, as mentioned previously, Lindau and Dandy have collected many isolated cases of angioma from the literature.

Autopsy revealed in the same patient here reported, multiple intracranial angiomas and multiple meningiomas. Unfortunately the spinal cord and meninges were not examined to find out if simi-

brain with dura weighed 1255 gm. Without further examination the brain and dura were suspended in 10 per cent neutral formalin. On frontal sectioning of the brain another large meningioma 4 by 3.5 by 4 cm. was found protruding into the right lateral ventricle. A detailed study of these multiple meningiomas will be reported in another paper.

When the dura mater was removed from the brain, after fixation, these meningioma nodules produced corresponding depression in the brain substance. The convolutions were markedly flattened and the sulci shallowed. The brain stem and the cerebellum showed a marked pressure cone.

The angiomatous nodules all occurred in the right frontal lobe, the smaller ones in the gray matter and the larger extending into the white substance. Located in the medial frontal gyrus 2.5 cm. from the medial fissure, there was a firm, circumscribed angioma 8 by 8 by 5 mm. Nearby, 5 mm. away were two other minute angiomas each about 1 mm. in diameter, lying wholly in the gray matter. In the marginal gyrus 3 cm. from the anterior frontal pole there were two more, measuring 7 by 10 by 4 mm., and 6 by 7 by 2 mm. respectively. In the same frontal section 1 cm. laterad from the median fissure, there were two others 5 by 7 by 2 mm., and 3 by 3 by 2 mm. in the cortex of the superior frontal gyrus. Further back in the marginal gyrus, 6 cm. from the anterior frontal pole and 5 mm. from the median fissure, there was still another angiomatous nodule 8 by 8 by 7 mm. The last figure in all the above dimensions represents the depth to which these tumor masses had penetrated the brain substance. These nodules produced on the external surface of the brain at the most only a very slight plateau-like elevation (Figs. 1, 2 and 3).

### MICROSCOPIC EXAMINATION

Sections from the various parts of the brain were stained with hematoxylin and eosin, Van Gieson's picric-acid fuchsin, Mallory's phosphotungstic acid hematoxylin, Weigert's resorcin fuchsin, Verhoeff's elastic tissue stain, Foot and Mènard's method for reticulum,<sup>3</sup> and Penfield's combined method for oligodendroglia and microglia.<sup>4</sup>

lar tumors were present there also. Wohlwill<sup>11</sup> and Schuback<sup>12</sup> separately reported the same case wherein a solitary angioma of the roof of the fourth ventricle was associated with an angiomatous tumor of the dorsal spinal cord. In the cases of Ohlmacher<sup>13</sup> and Tannenberg,<sup>14</sup> in addition to the multiple cranial angiomas, similar tumors were found in the spinal cord. Ohlmacher's case is of further interest in that the two cranial angiomas were also associated with a solitary cranial meningioma. Sands<sup>15</sup> reported a case of an angioma and a meningioma in one frontal lobe.

As seen in Table I, we have been able to find in the literature thirteen cases of multiple cranial angiomas. We consider Dandy's two cases (Nos. 6 and 7, reported by him as multiple angiomas) and Jaffé's case<sup>25</sup> as multiple telangiectases from their description and illustrations. Kalischer<sup>26</sup> described a case of multiple telangiectases of the left cerebral hemisphere, associated with a congenital telangiectasis of the left side of the face. Sachs<sup>27</sup> stresses this association and has found in the literature that the term *angioma racemosum arteriale* or *venosum*, had been applied to conditions which were not actually newgrowths. He suggested the name of intracranial telangiectasis for this condition. In our collected cases of multiple angiomata there were eight males to five females, with no sex mentioned in one case. The ages ranged from 14 to 81 years. No definite conclusions as regards the sex and age incidence can be drawn from such a small series. The multiplicity of these tumors varied from two to numerous. There appeared to be no site of predilection. Out of these fourteen collected cases, 8 or 57 per cent were associated with congenital anomalies or other types of newgrowths — multiple nevi of the skin, von Hippel's angioma of the retina, cavernomas of the liver, spleen and kidney, multiple cysts of the pancreas and kidney, one or more hypernephromas, one or more meningiomas, spinal osteoma, syringomyelia and hydrocephalus. Lindau has found that 20 per cent of the cases of von Hippel's disease have also an intracranial angioma. In my paper on multiple meningiomas<sup>28</sup> 45 per cent of the collected cases were associated with tumors of the acoustic nerve or generalized neurofibromatosis. This multiplicity in number and types of anomalies or newgrowths strongly suggests a congenital or dysontogenetic disturbance of the mesodermal germ layer. Microscopically, most of these tumors have been cavernomas. A

TABLE I.

Num- ber	Author	Sex	Age in years	Number of angiomas	Location of angiomas	Type of angiomas	Associated findings
1	Ohlmacher <sup>13</sup>	M	Adult	3	Callosal gyrus, optic thalamus, spinal cord.	Cavernous	Cranial meningioma, spinal osteoma.
2	Creite <sup>16</sup>	F	21	8	Both hemispheres, pons, cerebellum.	Cavernous	None
3	Finkelburg <sup>17</sup>	M	14	2	Corpora quadrigemina, 4th ventricle extending into cord.	Cavernous	Cyst of cord, hydrocephalus.
4	Koch <sup>18</sup>	M	47	2	Cerebellum	Cyst with angioma in wall	Diabetes, lymphangioma of pan- creas, cysts and fibroma of kid- ney, multiple adrenal rests in kidney, cavernoma of liver, ca- pillary and cavernous angio- mata of cord.
5	Huebschmann <sup>19</sup>	M	39	About 30	Both hemispheres	Cavernous	None
6	Friedrich and Stiehler <sup>20</sup>	M	41	2	Cerebellum, medulla.	Cyst with angioma in wall	Multiple cysts of pancreas, hydrocephalus.
7	Müller <sup>21</sup>	M	17	2	Caudate nuclei	Angioma arteriale racemosum	None
8	Malamud <sup>22</sup>	F	52	Numerous	Infundibulum, pallidum, pons-oblongata, both hemispheres.	Cavernous	None
9	Lindau <sup>1</sup>	M	32	Numerous	Cerebellum, medulla.	Cyst with angioma in wall, also capillary.	Hypernephroma, hydrocephalus.
10	Lindau <sup>1</sup>	..	..	3	Pons, cerebellum.	Cavernous	None
11	Fedoroff and Bogorad <sup>23</sup>	F	23	3	Frontal lobe, parietal lobe.	Cavernous	Cavernous angioma of spleen and kidney, breasts not de- veloped, no menses.
12	Kufs <sup>24</sup>	M	81	Numerous	Both hemispheres, right pyramid, pons.	Cavernous	Multiple vascular nevi of skin, multiple cavernomas of liver, abnormal position of left adrenal.
13	Tannenberg <sup>14</sup>	F	34	Numerous	Cerebellum	Capillary	Angiomas of cord and adjacent spinal ganglion, angiomatosis of liver, cysts of pancreas and kidney, syringomyelia.
14	Here reported	F	34	Numerous	Frontal lobe	Capillary	Multiple cranial meningiomata

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22. Malamud, W. Ueber einen Fall von multiplem Hämangiom des Zentralnervensystems mit bemerkenswertem klinischen Verlauf. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1925, 97, 651-671. This same case was presented before the Deutsche Verein f. Psychiat. u. Gesell. Deutsche Nervenärzte, Sept. 25, 1924, by A. Jakob. Ueber einen seltenen Fall von multiplem Hämangiom des Zentralnervensystem mit bemerkenswerten psychoneurologischen Symptomen. *Zentralbl. f. d. ges. Neurol. u. Psychiat.*, 1925, 40, 118-119.
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few were of the capillary type. In others, the main tumor mass consisted of a cyst without epithelial lining, in the wall of which there was an angiomatous nodule.

### SUMMARY

1. A case of multiple intracranial angiomas associated with multiple intracranial meningiomas all on the right side, is reported with autopsy findings.
2. The patient presented no symptoms or signs of definite cerebral localization.
3. The literature on the subject is briefly reviewed.

NOTE: My thanks are due Dr. John A. Sampson for his kind permission to use the clinical history of this case, and Dr. Victor C. Jacobsen for his help and advice during the preparation of this paper.

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## DESCRIPTION OF PLATES

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### PLATE 56

- FIG. 1. Frontal section of the frontal lobe of the brain, looking from behind. Arrow points to a small angioma. The deep depression on the lateral surface is due to the presence of a meningioma on the corresponding inner surface of the dura.
- FIG. 2. Two pieces from the mesial aspect and from the anterior pole of the right frontal lobe. The arrows point to four angiomas.
- FIG. 3. Photomicrograph, Van Gieson stain. The large angioma produces very little elevation of the brain surface. Two other minute angiomas are present in the cortex (arrows).  $\times 6.4$ .

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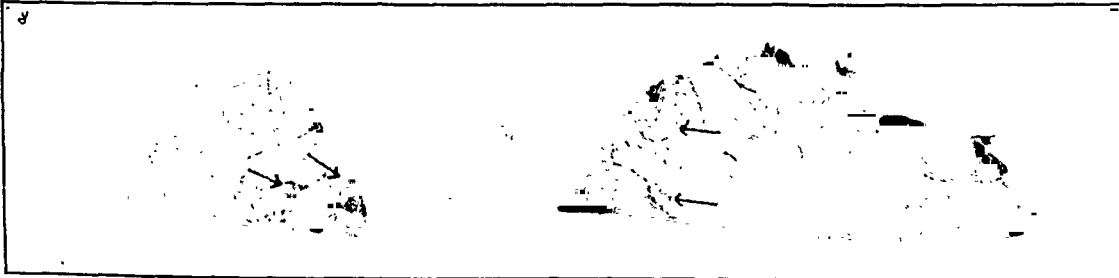
PLATE 57

FIG. 4. Photomicrograph, Penfield stain of a frozen section taken from the near vicinity of an angioma. Note the presence of numerous capillaries, their thickened walls and the highly branching forms.  $\times 140$ .

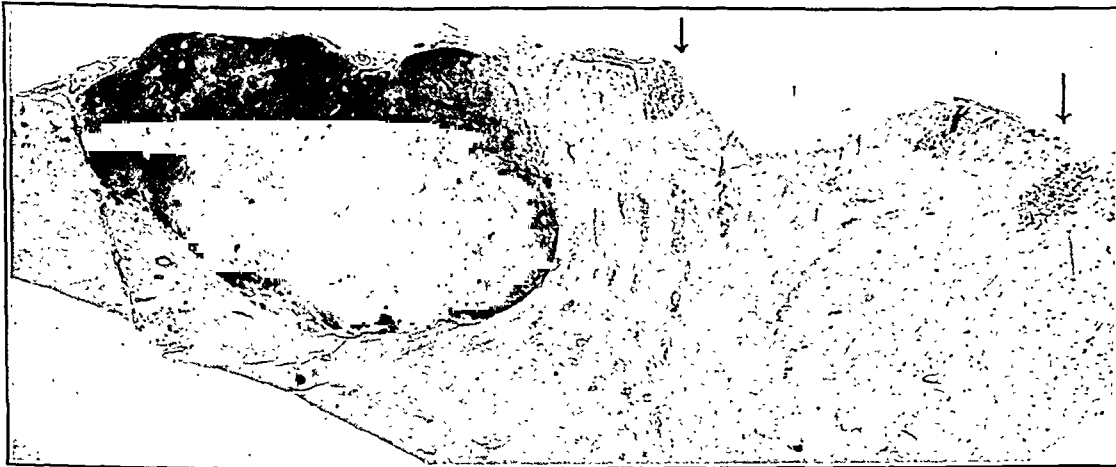
FIG. 5. Photomicrograph, Penfield stain of a frozen section taken from the edge of an angioma to show the capillary nature of the tumor in the brain cortex.  $\times 120$ .



I



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4



5

medulla, terminal bronchopneumonia, healed pulmonary tuberculosis of both apices, caseous tuberculosis of the right pulmonary lymph nodes, emphysema of the lungs, chronic adhesive pleuritis on the right side, atheroma of the aorta, mildly arteriosclerotic kidneys and recent laparotomy wound for cholecystectomy.

*Head:* The scalp was normal and stripped easily from the cranium. The skull cap and base were not remarkable. The middle ears were normal. The dura was tense over the brain. The spinal fluid was clear. Many opacities of various sizes could be seen through the external surface of the dura over the frontal, parietal and temporal regions. Brain with dura weighed 1255 gm. Brain and dura were suspended in 10 per cent neutral formalin.

On lifting away the dura from the brain cortex it was seen that the opacities mentioned previously were multiple, hard, sessile nodules and plaques attached to its inner surface, varying in size from 2 by 3 mm., to 13 by 18 by 10 mm. As shown in Fig. 1, these were present only in the right half of the dura. A total of thirty-eight nodules and plaques were counted. The largest nodule was attached to the inner surface of the dura in the region of the right Sylvian fissure. These dural nodules produced depressions on the corresponding surfaces of the brain. The convolutions appeared markedly flattened and the sulci shallowed. In the right hemisphere in the occipitoparietal lobes occupying the body and posterior cornu of the lateral ventricle there was found on frontal sectioning a firm, nodular growth 4 by 3.5 by 4 cm., of fibrous, gritty consistency (Fig. 2). The right chorioid plexus was strongly adherent to the inferolateral angle of the tumor. At first glance this tumor appeared to originate in the base of the brain, pushing into the ventricle and being easily enucleable. Microscopic sections and further study, however, showed that the growth was entirely covered by compressed brain tissue, which was of paper thinness just beneath the pia. The whole right striate body was pushed forward so that its frontal pole was 1.5 cm. anterior to that on the left side. The lateral ventricle on the left side was not dilated. The brain stem and cerebellum showed a marked pressure cone. The cerebral cortex of the right frontal lobe showed multiple small angiomas which are reported in another paper.

*Microscopic Examination:* Sections were stained with hematoxylin and eosin, Van Gieson's picric-acid fuchsin, Mallory's

MENINGIOMAS \*  
WITH SPECIAL REFERENCE TO THE MULTIPLE  
INTRACRANIAL TYPE

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INTRODUCTION

Multiple meningiomas and solitary meningioma with hyperostosis cranei are of interest because of their infrequent occurrence. The histogenesis of these tumors is still much disputed. Additional cases in which detailed histological studies have been made may throw some light on the origin of these growths.

CASE REPORTS

CASE I

*Clinical History:* M. H., female, white, 34 years of age, was admitted in 1928 to the service of Dr. John A. Sampson, Albany Hospital, for cholecystectomy and freeing of numerous dense adhesions around the gall bladder.

As regards the pertinent facts of her past history she had had no fits of anger, hysterical spells or domestic difficulties. However, she had been suffering from periods of depression for the past three years, lasting one to two days. When depressed she cried a great deal. Because of these depressive states the patient was advised to enter a state institution for observation but she refused to sign voluntary papers of commitment. Also, she had been having occasional dizzy spells and the feeling of pressure in the head. The knee jerks were equal. No clonus was elicited and the Babinski, Kernig and Romberg signs were negative. Complement-fixation tests for syphilis on the spinal fluid were negative.

Twenty-four hours after operation an acute respiratory paralysis suddenly developed. She responded to artificial respiration and went on breathing naturally for a few minutes but stopped breathing again. As her cardiac function continued apparently undisturbed, further artificial respirations were heroically attempted for at least two hours but with no avail. This was a striking clinical demonstration of the independence of the cardiac and respiratory centers in the medulla.

*Autopsy Findings:* Autopsy performed one hour after death showed the following: Multiple dural meningiomas, multiple angiomas of the cerebral cortex, a pressure cone of the cerebellum and

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cell clusters and whorls, indistinguishable histologically from those large whorls found in the various tumor masses.

In the section from the cerebral cortex, compressed by one of the larger dural tumor nodules, all the cortical vessels stand out prominently, due to a marked hyaline fibrotic thickening of the capillary wall. The perivascular Virchow-Robin spaces are widely distended.

In sections of the tumor masses stained with Van Gieson's picric-acid fuchsin, the pink-staining collagen fibrils are seen to permeate the tumor, occurring either as single strands or as dense thick masses and whorls. In other areas the broad bands of collagen produce a sort of honeycombed branching network, in the interstices of which one or more tumor cells lie. The collagen is present only around the blood vessels in the very cellular areas. In the smaller dural tumors collagen appears only as isolated, mostly short, thickened pink strands or nodes. The intimate connection between these tumors and the dura is even more noticeable in sections stained by this method. In some sections it appears as if the tumor cells have encircled each separate collagen component of the dura, producing numerous whorls with hyaline centers.

Weigert's resorcin fuchsin and Verhoeff's elastic tissue stains both show that elastic fibers are scarce in sections of the various tumor masses. None are found in the very cellular areas. In those areas where spindle cells predominate the elastic fibers appear as short thick strands or longer dark threads. In the collagenous areas these elastic fibrils run through the collagen bundles as long thick strands.

Mallory's phosphotungstic acid hematoxylin stain shows many fibroglia fibrils in the spindle cell areas. Reticulum, as stained by the Foot and Ménard method,<sup>1</sup> is absent in the very cellular areas of the large polyhedral cells; is abundant in the spindle-shaped cell regions (Fig. 5), and scarce in the very collagenous portions of the tumors.

In sections stained by Penfield's combined method<sup>2</sup> there is no demonstrable increase in size and number of the microglia and the oligodendroglia in the immediate vicinity of the large ventricular meningioma, nor in the cortex depressed by the numerous dural growths. However, a reactive increase in neuroglia fibrils is present in the brain tissue surrounding the ventricular tumor.

phosphotungstic acid hematoxylin, Weigert's resorcin fuchsin, and Verhoeff's elastic tissue stain; in addition, Foot and Mènard's method for reticulum,<sup>1</sup> and Penfield's combined method for oligodendroglia and microglia<sup>2</sup> were used. As the brain had been fixed in neutral formalin the sections were first mordanted in potassium bichromate for several hours whenever necessary.

Sections from the ventricular tumor, stained with hematoxylin and eosin, show everywhere irregular interlacing bands of spindle cells with oval vesicular nucleus. Much collagen is present with a tendency toward whorl formation. In these collagenous areas there are calcific deposits, spiculated, sometimes irregularly oval, showing no concentric lamellations. Toward the periphery there are many masses of large polygonal endothelioid cells with large, oval to almost round vesicular nucleus. Many of these cells are arranged in whorl formation by themselves or around capillaries. Those cells forming larger whorls have become spindle-shaped. In still older whorls a few collagen fibrils are laid down between the cells. Much older whorls consist mainly of a lamellated fibrous tissue core. Some of these hyaline fibrous balls contain irregular calcium deposits in the center. Finally, psammoma bodies showing concentric calcification are most numerous in and around these cellular areas (Fig. 3). Along one edge, in close apposition to and adherent to the main tumor mass, the fibrotic but still vascular and tufted remains of the chorioid plexus can be distinguished. Most of the capillaries here are almost occluded, due to the thickened hyalinized fibrous wall. The remaining capillaries are widely dilated, appearing like venous sinuses. In the plexus there are a few calcified psammoma bodies and minute cellular clusters and whorls, which in serial sections show no connection with the main tumor (Fig. 4).

Sections taken from the dural tumors *en plaque* and of the nodular type all show a histological picture similar to the previous tumor — interlacing bundles of spindle-shaped cells with a tendency to cellular and fibrous whorl formation, collagen fibrils and psammoma bodies. The very small tumors *en plaque* contain only multiple minute cellular and fibrous whorls not yet calcified. These tumor masses are intimately connected with the dura. The dense collagenous fibers on the inner aspect of the dura are seen to fray out into the tumor tissue. A section through the region of the pachionian granulations near the superior sagittal sinus shows many



cranei. Here the calvarium is 20 mm. thick as compared with the 6 mm. of the opposite side. The external table is but very slightly elevated. The tumor apparently started near the midline compressing the brain caudad and laterad (Fig. 6). It extends 2 cm. from the frontal lobe anteriorly to the level of the Rolandic fissure posteriorly. There are numerous pacchionian granulations on each side of the falx cerebri in the vicinity of the growth. The largest of these measures 5 by 5 by 2.5 mm., being about 1 cm. away from the posterior pole of the growth, and having grossly the same appearance as the large tumor. Sulci and convolutions of the cortex do not appear shallowed or flattened. There is no cerebellar pressure cone. The cut surface of the growth after fixation is very fibrous, gritty and pearly gray. The edge of the tumor is sharply demarcated. Except for some filmy strands it can be easily shelled out. The left lenticulostriate body is not involved. The lateral ventricles are not very much compressed nor dilated, the anterior horn on the same side as the tumor being somewhat larger. Posteriorly the tumor penetrates the gray matter of the precentral gyrus over an area 2 mm. in diameter and 17 mm. from the median fissure, being adherent to the overlying pia-arachnoid.

*Microscopic Examination:* All the different staining methods mentioned under Case 1 were again employed here. The microscopic pictures after the various staining reactions are identical with those of Case 1. In Fig. 7, the invasion of the bone by the tumor cells is clearly shown.

Sections of the pacchionian granulations show many cellular nests and whorls. The chorioid plexuses contain many psammoma bodies and cell nests.

In the sections from the compressed brain the perivascular spaces of Virchow-Robin are widely distended. Numerous minute, spherical, partially calcified "amyloid" bodies are present here and there in the cortex; many are present in the endothelial lining cells of the cortical capillaries.

## DISCUSSION

Meningiomas may occur as a diffuse process, or may be single or multiple. The very rare diffuse form of meningioma or meningiomatosis has been reported under a variety of diagnoses by Bassoe and Shields,<sup>3</sup> Markus,<sup>4</sup> Greenfield,<sup>5</sup> Pirie,<sup>6</sup> Cassirer and Lewy,<sup>7</sup>

## CASE 2

*Clinical History:* F. G., laborer, white, 45 years of age, was admitted in 1929 to the service of Dr. George S. Amsden for convulsions. He had had these epileptiform seizures for the past nineteen years. Since onset they had been more or less typically the same. First he moaned, then the right side of his face pulled up, his head turned to the right and his right arm began to jerk. He lost consciousness, frothed at the mouth, bit his tongue and was incontinent of urine and feces during an attack. When he came out of an attack he usually appeared perfectly normal. For many years these attacks had been occurring about once every two months. Soon they began to occur every day, and for the last two or three days he had had one attack every twenty minutes.

The physical examination showed a well developed and nourished man lying in bed and having an occasional convulsion. The order of events during one of these convulsions was: twitching of both sides of the face, mouth pulled over to the right, head and eyes pulled over far to the right, and then the right arm began to jerk clonically. The whole thing was over in about one to two minutes. During the attack the pupils did not react, the teeth clamped down and the sphincters relaxed.

There was a complete smoothing out of the lines on the right side of the face. The lips could not be drawn up. The forehead wrinkled equally well on both sides. Extra-ocular movements were normal. Fundi had been consistently negative. Tongue protruded with marked deviation to the right. Reflexes were diminished throughout but were apparently equal. No muscle weakness was elicited. There were varicose veins with an old varicose ulcer on the left leg.

The first and last 5 cc. tubes of the spinal fluid were perfectly clear but the middle tube showed definite xanthochromia. The initial pressure was 350 mm. water; bilateral jugular compression gave a prompt rise to 700 mm. and back to 300; right jugular compression gave a prompt rise to 350 mm. and back to 300; left jugular compression gave a prompt rise to 350 mm. and back to 300. After 5 cc. of the spinal fluid were removed, the pressure fell to 220 mm.; after 10 cc. removal, 190 mm.; after 15 cc. removal, 150 mm.; after 20 cc. removal, 120 mm.; and after 25 cc. removal, 100 mm. Cell count gave 7 cells per cmm. with a differential of 90 per cent lymphocytes. Pandy, Takata-Ara, Kahn and Wassermann tests of the spinal fluid were all negative.

During his seven days stay in the hospital the patient continued to have convulsions in spite of medication. He had as many as 100 convulsions during the course of twenty-four hours. At night he might have 50 convulsions. He seemed not to become exhausted, the convulsions being very light in character. He became gradually worse and his breathing labored. The left lung showed beginning hypostasis. Temperature rose to 108, his pulse to 167, whereas his respirations which were 55 the day before fell to 48 about forty-five minutes before death.

*Autopsy Findings:* Autopsy performed one and one-fourth hours later is essentially not remarkable, excepting in the calvarium and the brain. In the left superior frontoparietal region near the midline the dura is strongly adherent to the internal surface of the skull cap over an area about 4.3 cm. in diameter. The inner table is invaded by a nodular tumor 8.7 by 6.4 by 4.8 cm., producing a hyperostosis

As seen in Table I, we have been able to find in the literature only twenty-two cases of multiple cranial meningiomas, including the one here reported. Globus <sup>49</sup> before the New York Neurological Society in 1928 mentioned that there was only one instance of multiple meningiomas out of a personal experience of 120 to 130 tumors but

TABLE I

Number	Author	Age in years	Sex	Number of primary meningeal tumors	Associated findings
1	Wishart <sup>30</sup>	21	M	Numerous	Bilateral acoustic tumors and multiple neurofibromatosis
2	Langdon <sup>34</sup>	32	F	Numerous	Unilateral acoustic tumor
3	Henneberg and Koch <sup>35</sup>	26	M	Numerous	Bilateral acoustic tumor
4	Schmidt <sup>36</sup>	77	M	2 tumors	None
5	Schmidt <sup>36</sup>	88	..	2 tumors	None
6	Fraenkel and Hunt <sup>37</sup>	42	F	Over 100 tumors	Bilateral acoustic tumors
7	Westphal <sup>38</sup>	26	F	Numerous	Bilateral acoustic tumors and multiple neurofibromatosis
8	Verocay <sup>39</sup>	31	M	Numerous	Bilateral acoustic tumors and multiple neurofibromatosis
9	Henschen <sup>40</sup>	25	M	Numerous	None
10	Leischner <sup>41</sup>	40	M	Numerous	Unilateral acoustic tumor
11	Heuer and Dandy <sup>42</sup>	Adult	M	2 tumors	None
12	Greenfield <sup>5</sup>	31	M	3 tumors	None
13	Symonds <sup>43</sup>	27	M	Numerous	Bilateral acoustic tumors and multiple neurofibromatosis
14	Krivy <sup>44</sup>	Old	F	3 tumors	None
15	Firket <sup>45</sup>	27	F	10 tumors	Bilateral acoustic tumors and one spinal meningioma
16	Casper <sup>11</sup>	Old	F	Numerous	None
17	Casper <sup>11</sup>	52	F	Numerous	None
18	Casper <sup>11</sup>	72	M	Numerous	None
19	Savy <i>et al.</i> <sup>46</sup>	39	F	2 tumors	None
20	Régnier <i>et al.</i> <sup>47</sup>	36	M	2 tumors	Bilateral acoustic tumors and multiple neurofibromatosis
21	Flick <sup>48</sup>	54	F	Numerous	None
22	Here reported	30	F	38 tumors	Multiple angiomata

he did not state whether the multiple condition was intracranial, intraspinal, or both. In the collected series there were eleven males to ten females with no sex data in one case — apparently no predilection for either sex. There is a wide variation in the age incidence as the youngest was 21 and the oldest, 88 years of age. There were

Moorhead and Wigham,<sup>8</sup> Fried,<sup>9</sup> Connor and Cushing,<sup>10</sup> and Casper.<sup>11</sup> Also, meningiomas may be histologically malignant. Craig<sup>12</sup> found eleven cases among fifty-six cases of intracranial meningiomas. Bailey<sup>13</sup> reported two cases of meningioma of the fibrosarcomatous type. Towne's<sup>14</sup> second case showed a meningioma of the falx cerebri with invasion of the inferior longitudinal sinus, extension into the straight, superior longitudinal, right and left lateral sinuses, jugular and innominate veins and superior vena cava.

Numerous instances of solitary meningiomas with or without cranial hyperostosis have already been reported by Cushing<sup>15, 16, 17, 18</sup> and his associates,<sup>19, 20</sup> Tooth,<sup>21</sup> Greenfield,<sup>5</sup> Alurralde and Sepich,<sup>22</sup> Holmes and Sargent,<sup>23</sup> Cordes,<sup>24</sup> and others. This hyperostosis cranei (hemicraniosis of the French) has been interpreted as being due to an osteoblastic invasion of the skull by the tumor (Penfield,<sup>25</sup> Phemister,<sup>26</sup> Sosman and Putnam,<sup>27</sup> and others), or as resulting from a defensive reaction of the bone to the progressing dilatation of the blood vessels (Kolodny<sup>28</sup>). In a study of ten cases of meningioma with cranial changes, Kolodny found that proliferation of bone precedes the infiltration of the skull by tumor cells. Spiller<sup>29</sup> also believes that the enlargement of the bone may come first. Wishart's case<sup>30</sup> is uniquely interesting in that there were two hyperostoses cranei, one of which had become perforated by the invading tumor. Also, in the cases of Mix<sup>31</sup> and of Dumas and Dechaume,<sup>32</sup> the dural growth penetrated the skull out into the scalp. The growth in Alurralde and Sepich's case was strongly adherent to the base of the skull in the right middle fossa. In Daspit's second case,<sup>33</sup> the dural meningioma perforated the temporal and sphenoid bones and extended down the neck and into the right sphenoid and posterior ethmoid sinuses. That all tumors perforating the vertex of the skull may not be due to a meningioma is shown by Daspit's first case of a perforating gliosarcoma. Cushing found that the bony thickening of the cranial vault is more apt to be much more pronounced in meningiomas *en plaque* than in the more massive type with a relatively small area of meningeal attachment. He has found them palpable externally, or demonstrable by X-ray in at least 25 per cent of the meningiomas. Furthermore, tumors parasagittal in origin and those arising from the temporo-frontal meninges, adjacent to the Sylvian cleft, seem to be accompanied by hyperostosis cranei more often than those in other situations.

dural endothelioma does not arise from the dural cellular elements but is of arachnoidal origin, either from the pacchionian granulations or from these cell clusters. Later, Weed, Winkelman and Wilson also pointed out the possible relationship of these cell nests to true meningiomas. Indeed, these cell whorls and clusters may be called miniature meningiomas as they correspond histologically to the fully developed meningeal tumor.

But what is the parent cell of this meningioma group of tumors? Their histogenesis is still a mooted question. Cushing suggested the name "meningioma" in view of the fact that its origin is still a disputed point and that the name is non-committal as regards its genesis from a particular type cell. Mallory<sup>57</sup> pointed out its fibroblastic nature by finding that elastic fibrils are elaborated by these tumor cells. Crowe and Jones<sup>58</sup> and Van Wagenen<sup>59</sup> confirmed Mallory's findings. However, Bailey<sup>14</sup> warns us that though in rare instances the cells of a meningioma may form fibroglia, collagen and elastin, this does not prove they are not endothelial cells. Maximow<sup>60</sup> watched in tissue cultures of the leptomeninges endothelial cells from the severed ends of small arteries growing out, forming side processes and losing their differences from fibroblasts. Kredel<sup>61</sup> noted that in young tissue cultures of meningiomas the typical cells seemed to be a mononuclear-macrophage series rather than fibroblasts, whereas in older cultures the cells appeared gradually to assume a form more closely resembling that of fibroblasts. More recently, Buckley and Eisenhardt<sup>62</sup> from tissue culture studies stated that the meningioma tumor cells which grew out in a reticular pattern are mesodermal, non-phagocytic cells. They state that these growing tumor cells did not appear to be of the fibroblastic type. On the other hand, Mallory and Parker<sup>63</sup> from histological study conclude that reticulum is collagen in separated form rendered prominent by the silver stain, that all collagen is produced by fibroblasts, that there are no reticular cells other than fibroblasts and that endothelial cells do not produce an intercellular substance. Yet Foot<sup>64</sup> has found much reticulum in a malignant endothelioma originating in the reticulo-endothelium of lymphoid tissue. To quote from him, "the morphological differences between vascular and reticulo-endothelium may, after all, be merely an evidence of a difference in the physiology of the same cell due to varying reactions to varying surroundings." Indeed, Piney<sup>65</sup> and more recently

no other cranial findings in eleven cases; two had an associated unilateral tumor of the acoustic nerve, eight had in addition bilateral tumors of the acoustic nerve; and four of these last eight cases also showed multiple neurofibromatosis of the von Recklinghausen type of the cranial and peripheral nerves. Casper's third case is particularly interesting in that the two primary meningeal tumors were associated with a diffuse meningiomatosis of the pia-arachnoid and an endothelioma of the duodenum. In no case was the multiple nature of the meningiomas suspected before death.

Other interesting cases of multiple cranial and spinal tumors, though not strictly germane to this study, may be mentioned. Biggs' case<sup>50</sup> showed a bilateral tumor of the acoustic nerves and only one tumor of the falx cerebri. In a case reported by Schultze,<sup>51</sup> in addition to the cranial meningeal tumor there were multiple tumor nodules of the spinal pia, all histologically similar. In Funkenstein's case,<sup>52</sup> there were a psammoma and an osteopsammoma of the spinal dura associated with bilateral tumors of the cerebellopontine angle and multiple neurofibromatosis.

The frequent occurrence of multiple cranial meningiomas with bilateral acoustic nerve tumors and multiple neurofibromatosis has been regarded as being more than fortuitous by Verocay,<sup>39</sup> Cushing,<sup>53</sup> Firket,<sup>45</sup> and Régnier and his associates.<sup>47</sup> Both Verocay and Cushing interpret this in the light of some anomaly of development of the nervous system, whereas Régnier and his associates state that they have been able to find all transitions between the meningeal tumors and the tumors of the nerves. From Table I, it is seen that eleven, or 50 per cent of the cases were not associated with other types of tumor formation. This would suggest that the association of meningeal tumors and multiple neurofibromatosis is not an *a priori* relationship but an expression of the predisposition or susceptibility of the individual through hereditary and dysontogenetic influences.

It is to be noted here that solid cell clusters with a tendency to irregular massing of nuclei or formations of small whorls have been frequently noted in the arachnoid by Schmidt,<sup>36</sup> Le Gros Clark,<sup>54</sup> Weed,<sup>55</sup> and Winkelman and Wilson.<sup>56</sup> They occur at all ages from infancy on, but are an almost constant finding in the later decades of life. Weed found these cell clusters undergoing hyaline changes and calcification. Schmidt in 1910 first suggested that the so-called

become the endothelium of the primitive blood cells. These blood spaces join one another and thus form a network of channels which soon ramify throughout the entire embryo.

Thus, as seen from the embryology of the meninges and of the vascular system, we are concerned strictly with the mesoderm in regard to the origin of the tumors in our cases; the multiplicity of these dural and angiomatous newgrowths in our first case would indicate a disturbance of the mesodermal germ layer, probably congenital.

We believe that the large ventricular tumor in our first case arose from the mesodermal elements of the chorioid plexus, an unusual origin for a meningioma. In support of this we find that: (a) the chorioid plexus is strongly adherent to the tumor mass; (b) this plexus contains numerous psammoma bodies and, what is more important, cell nests or whorls which by serial sections are found to have no connection with the tumor itself and which histologically are indistinguishable from the cellular areas of the tumor; (c) the tumor is surrounded on all sides by brain tissue, being wholly located in the lateral ventricle.

One would expect from the large size of the tumors in both of our cases, producing much pressure atrophy of the adjacent brain tissue, that there would be some reaction on the part of the interstitial tissue of the brain. However, microglia and oligodendroglia are not significantly increased or decreased. There is no demonstrable swelling of the oligodendroglia. Neuronophagia is absent. Only a moderate reactive gliosis is present in the immediate neighborhood of the ventricular tumor. All this bespeaks an extremely slow tumor growth in the two cases. In Case 2, it is definitely known that the onset of symptoms began nineteen years before death. Furthermore, this extreme slow growth is indicated by the presence of numerous psammoma bodies and abundant collagen fibrils, with very few cellular areas in these tumors.

## SUMMARY

1. Two cases of cranial meningioma are here presented: the first, one of multiple dural meningiomas associated with multiple angiomatous of the cerebral cortex; and the other, a solitary meningioma with hyperostosis cranei.

Bailey, Cushing and Eisenhardt<sup>19</sup> believe that neoplastic endothelial cells may differentiate and form strands of reticulin so characteristic of these growths. In other words, a meningioma may belong to the reticulo-endothelioma group of tumors. We find that the tumor cells in both of our cases elaborate reticulum, fibroglia, collagen and elastic fibrils, all derivatives of mesodermal cells. Bailey, Cushing and Eisenhardt state that meningoblasts are capable of differentiation into angioblasts, fibroblasts, and possibly other types of cells. At any rate, tissue cultures and histological studies seem to indicate that the type cell of the meningioma is not fully differentiated, having endothelial and fibroblastic developmental tendencies which seems to depend on the stage of the differentiation, the younger, more cellular parts having endothelial characteristics and the older parts showing fibroblastic qualities.

It may not be amiss to review here briefly the embryology of the meninges. According to Wislocki,<sup>66</sup> the meninges in mammals arise as a differentiation of the middle germ layer about the neural tube.

The primitive mesenchyme condenses into two laminae, the outer of which becomes the dura, and the inner the leptomeninges. This inner lamina itself divides partially by a condensation of the mesenchyme, giving rise to the pia and arachnoid which remain connected with each other by a porous mesenchymal stroma. The subdural and subarachnoidal spaces are both lined by pavement-like mesothelial cells derived from the middle germ layer. Key and Retzius (1875), cited by Wislocki,<sup>66</sup> demonstrated by the silver impregnation method that the subdural space is lined by a continuous layer of mesothelial cells. The chorioid plexus arises from the membranous portion of the roof of the forebrain and hindbrain as a series of buds and folds, consisting of a richly vascularized connective tissue core derived from the periaxial mesenchyme, and a covering of epithelial cells, which represents a modified part of the ependymal lining of the ventricle. As regards the pacchionian granulations, Luschka (1855), cited by Wislocki,<sup>66</sup> first distinguished their origin from the arachnoid and named them "arachnoidal villi."

According to Bailey and Miller<sup>67</sup> the formation of blood vessels in all the higher vertebrates, including mammals, begins in the area opaca of the blastoderm. In this area the blood islands arise, being composed of rounded cells which have developed from the branched mesodermal (mesenchymal) cells. The superficial cells of an island



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2. The tumor cells of the meningioma are mesodermal in origin. They elaborate reticulum, fibroglia, collagen and elastic fibrils. The multiplicity of the intracranial meningiomas and the angiomas in Case 1 connotes a marked disturbance of the mesodermal germ layer.

3. Clinically and histologically, the meningiomas in these two cases indicate a long drawn-out course. Considering the large size attained by these growths it is remarkable that a brain tumor was not suspected. The first patient was thought to be a mental case due to her frequent depressive states associated with fits of crying. The second patient, who was under the constant care of a neurologist, was being treated for idiopathic epilepsy.

4. The brain tissue, immediately surrounding the meningiomas and the angiomas and also far removed from these growths, shows no reactive increase in microglia or oligodendroglia, although in the adjacent cerebral cortex of both cases, so-called "amyloid" bodies are very abundant. A reactive gliosis is present in the immediate neighborhood of the ventricular tumor.

NOTE: Grateful acknowledgment is made to Drs. John A. Sampson, George S. Amsden, and LaSalle Archambault for permission to use the clinical histories of the two cases here reported. I am very much indebted to Dr. Victor C. Jacobsen for helpful suggestions.

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## DESCRIPTION OF PLATES

### PLATE 58

FIG. 1. Dural cap, inner view, showing the multiple meningiomas in nodular and plaque formation.

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PLATE 59

FIG. 2. Large meningioma filling the right lateral ventricle and apparently arising from the chorioid plexus.

FIG. 3. Photomicrograph of a section from the lateral ventricle meningioma. Note the whorl formation and the many psammoma bodies.  $\times 126$ .

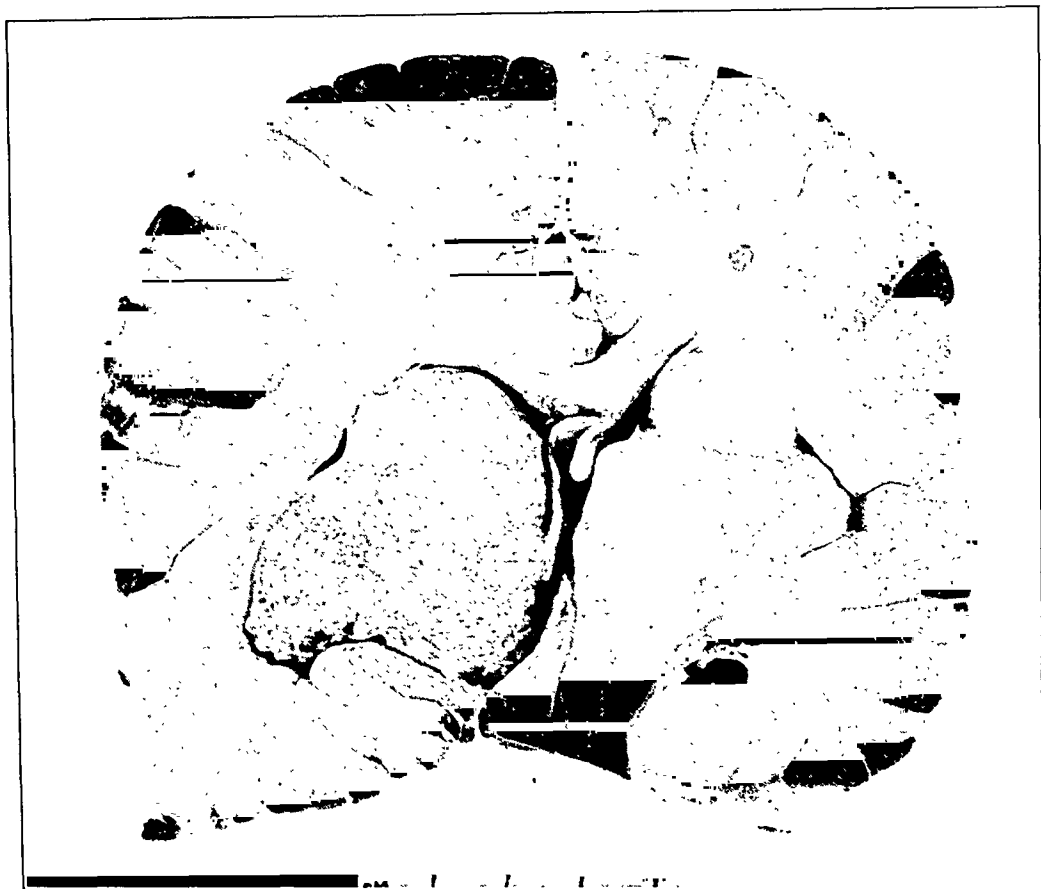


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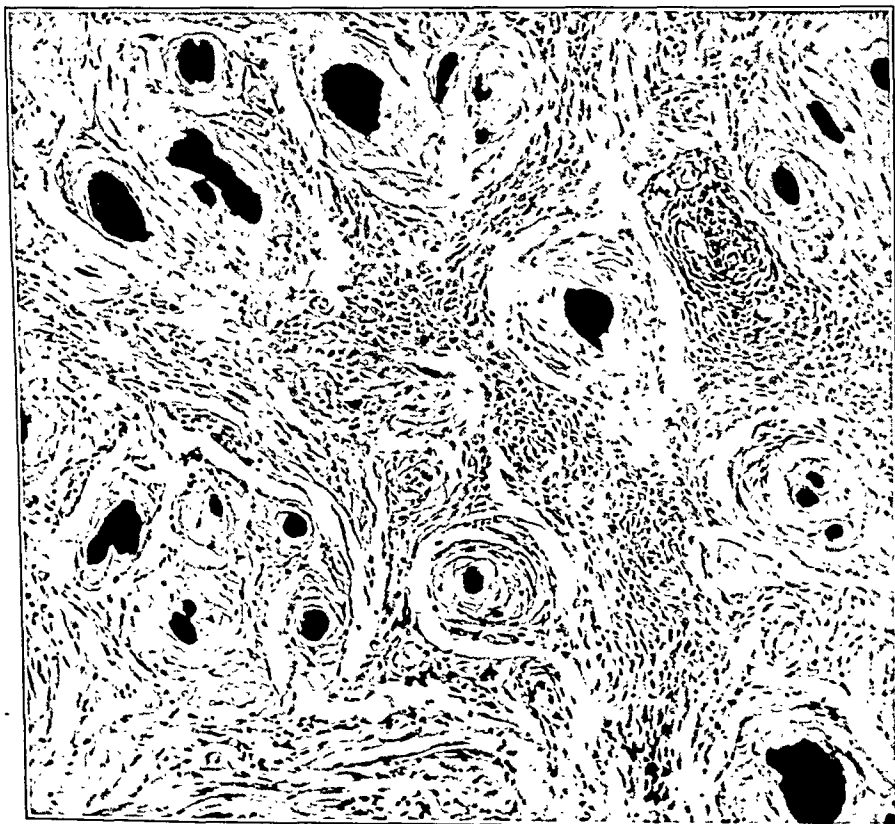
PLATE 60

FIG. 4. Photomicrograph of one of the cellular clusters found in the chorioid plexus of the right lateral ventricle. It forms a sort of a sheath around the vessel. It is histologically indistinguishable from the cellular areas of Fig. 3.  $\times 430$ .

FIG. 5. Photomicrograph, Foot and Mènard method for reticulum. Note the abundance of fine reticular fibrils in intimate association with the tumor cells.  $\times 625$ .



2



3



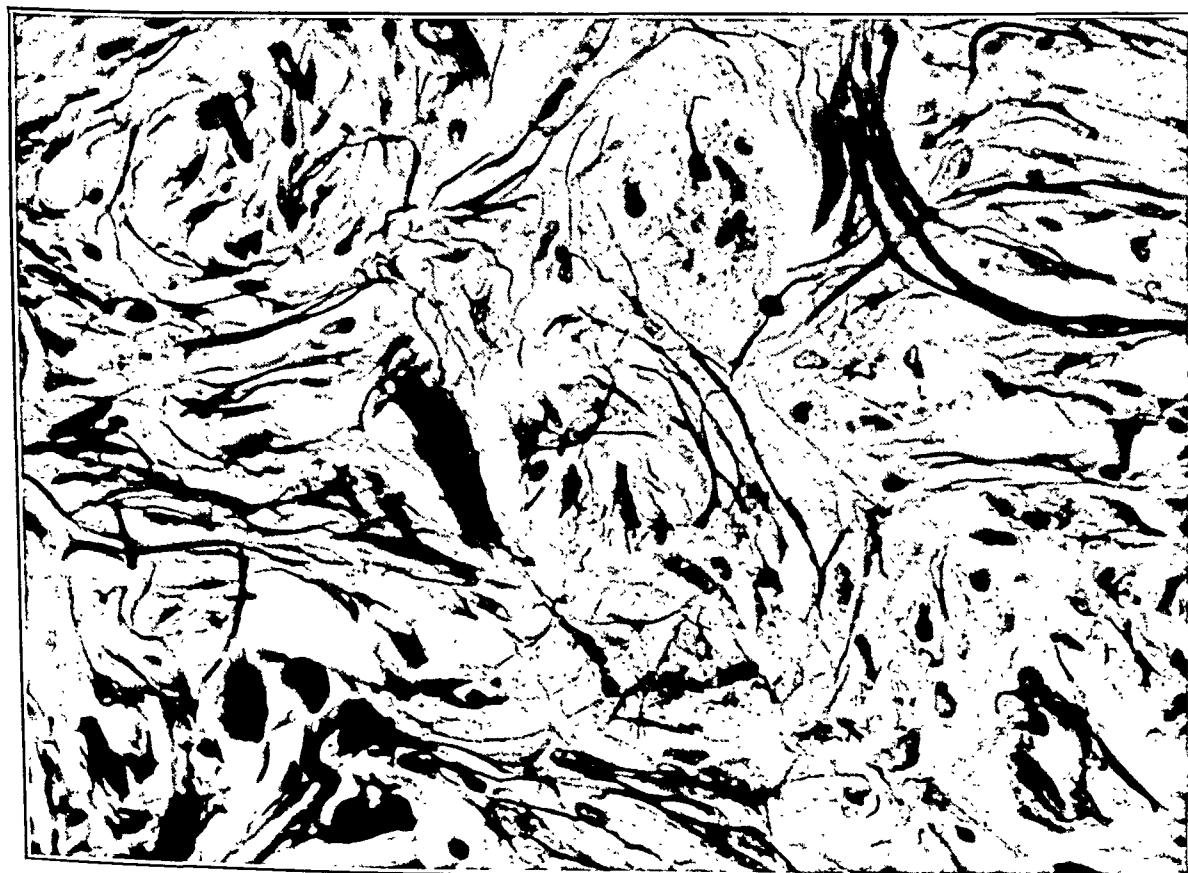
PLATE 61

FIG. 6. Frontal section of the left frontal lobe showing the marked destruction by pressure atrophy of this portion of the cortex and the production of hyperostosis cranei.

FIG. 7. Photomicrograph of a section through the hyperostosis cranei. Note the invasion of the diploic spaces by the tumor cells.  $\times 100$ .

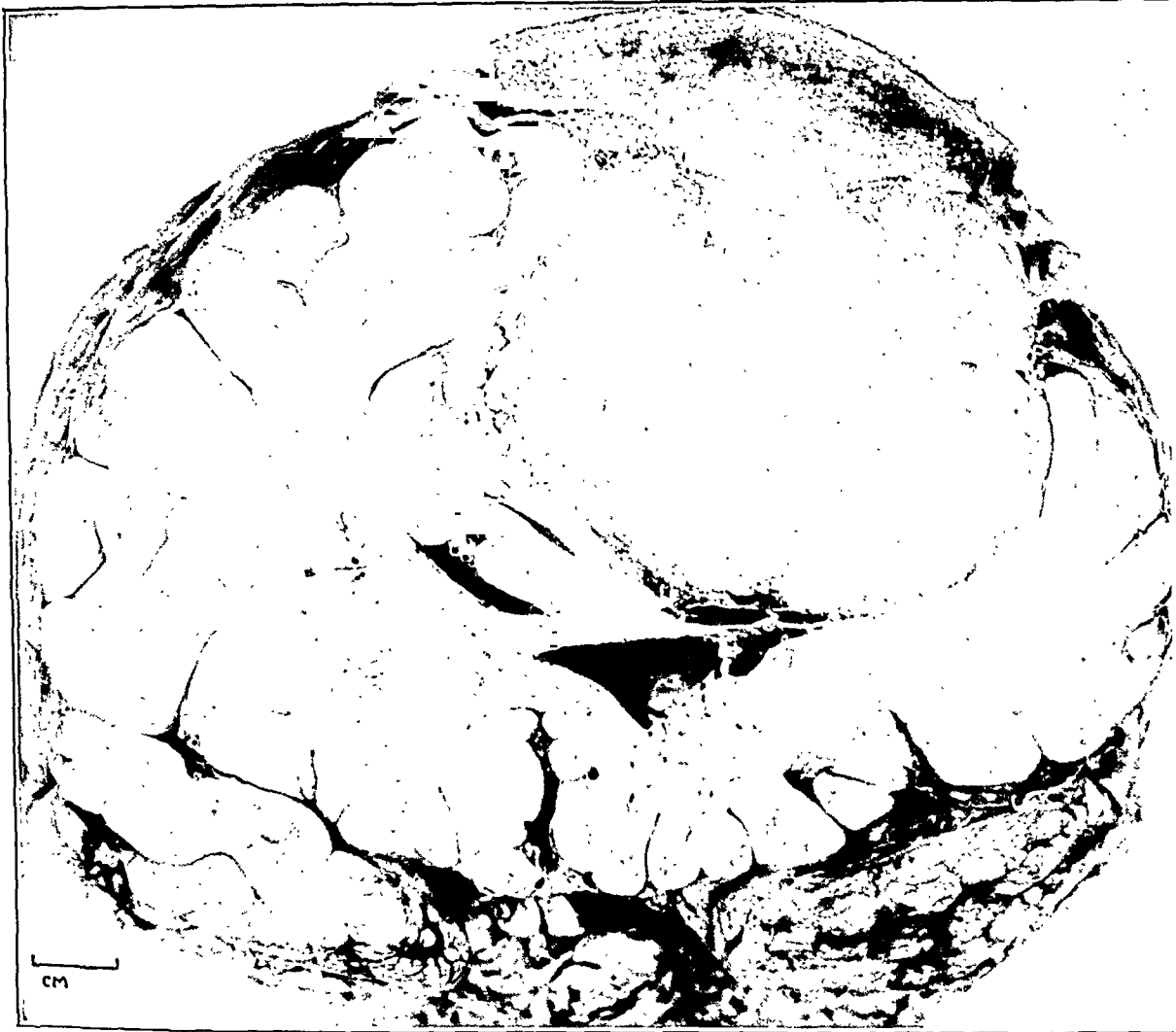


4



5





6



7

## MORPHOLOGY

The shape and relative size of the cytoplasmic inclusions in guinea pigs are well illustrated in the photomicrographs (Figs. 10 and 11) which were taken at a magnification of 2000 diameters. The inclusions are clearly visible in the distal parts of the cytoplasm between the nuclei and the lumina of the ducts. They are masses of variable size which in section tend to take a roughly oval shape with their long axes parallel to the cell membrane. This oval shape is represented also in Fig. 4 after the application of the Feulgen reaction.

Many attempts were made to dissect out inclusion-laden cells in order to study the inclusions in the fresh state without the aid of supravital stains, but none of them was successful. In permanent preparations the internal structure of the inclusions depends to some extent upon the technique used.

After fixation in Zenker's fluid and coloration by Giemsa's method the inclusions seem to be made up of clusters of tightly packed and very minute, strongly basophilic particles. That each inclusion mass, or clump of particles, is resilient and fairly dense is shown by the fact that when applied to the nuclear membrane they often indent it because the membrane appears to be more yielding than the inclusion. A good example of such nuclear indentation is shown in Fig. 11.

A wide variety of other fixatives reveals the same particulate appearance irrespective of the stain employed, because morphological characteristics are merely accentuated or suppressed but seldom modified by tinctorial procedures. With some, such as Giemsa's sublimate, the inclusions appear to be more homogeneous in structure. Frequently, and irrespective of the fixation, the centers of the inclusions are less dense than their peripheral parts; indeed the central areas may look like clear vacuoles being devoid of any detectable formed material. This is the appearance represented in Fig. 4. The inclusions are usually limited by a more or less distinct halo.

## MICROCHEMISTRY

These cytoplasmic inclusions are characterized by their great resistance to solvents. No ordinary fixative destroys them. They withstand 80 per cent alcohol, Carnoy's fluid which contains 30 per

## CYTOPLASMIC INCLUSIONS PRODUCED BY THE SUBMAXILLARY VIRUS \*

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It has been shown by Cole and Kuttner<sup>1</sup> and by Kuttner<sup>2</sup> that the cellular changes discovered by Jackson<sup>3</sup> in the submaxillary glands of a large proportion of apparently normal guinea pigs are not protozoan parasites but rather alterations caused by a filterable virus. The changes are characterized by marked cytoplasmic and nuclear hypertrophy and by the appearance in the nuclei of acidophilic inclusions which have a distinct resemblance to the intranuclear bodies described by Tyzzer<sup>4</sup> in the cutaneous lesions of varicella — a similarity which was first noted by Goodpasture and Talbot.<sup>5</sup> These intranuclear inclusions in the submaxillary glands of guinea pigs have attracted much attention because it has further been found that they look like inclusions caused by other well recognized viruses such as herpes and virus III, as well as still other nuclear inclusions which occur occasionally in human tissues in the absence of recognizable clinical symptoms (Goodpasture and Talbot, and VonGlahn and Pappenheimer<sup>6</sup>).

In this way attention has been focused on the nucleus to the almost complete neglect of the cytoplasm. This is illogical if we are to gain a true appreciation of the nature of the cellular response to the virus. A reaction is setting in and many cytological observations of fundamental interest have recently been made by Scott<sup>7</sup> and by Scott and Pruett.<sup>8</sup> There remain, however, certain conspicuous cytoplasmic inclusions which thus far have been largely ignored. They were first observed and clearly figured by Jackson who thought that they were "merozoites." Goodpasture and Talbot simply confirmed Jackson's discovery of their occurrence in guinea pigs' submaxillary glands. In the more recent studies of Cole and Kuttner, and of Kuttner, no reference is made to them. The purpose of this paper is to record detailed observations concerning them.

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in the adjacent greatly hypertrophied cell containing a large intranuclear inclusion and many smaller, spherical, cytoplasmic ones. The second kind of evidence was secured by making intraperitoneal transplantations of parts of submaxillary glands containing inclusions. This was done with three guinea pigs. The transplanted tissues were removed after forty-eight hours and prepared for study. The large cells containing inclusions were found to be unaltered whereas the other cells were in advanced stages of autolysis, which is consistent with the hypothesis that the former were dead to start with. This goes to show that the tinging with mucicarmine may not indicate the presence of mucus.

In the second place, if the inclusions do consist of mucus one would expect them to react in the same way to fixatives and stains as known mucus and granules of mucigen in other parts of the same sections of the salivary glands; but they do not. They are both more resistant to solvents in fixatives and more basophilic than mucus in neighboring cells.

Millon's reagent was then applied but no coloration of the cytoplasmic inclusions could be obtained which was sufficiently intense to justify any conclusion one way or the other.

The Feulgen reaction for thymonucleic acid was tried in accordance with the detailed instructions given by Cowdry<sup>11</sup> and the results are given in black and white in Figs. 3 and 4. The black parts represent the distribution of the deep purple color which is formed in substances said to contain thymonucleic acid. In the cell shown in Fig. 3 this purple color (now black) was limited to the nuclear membrane and to certain masses within the nucleus in close association with the large nuclear inclusion, as well as to the nucleus of the unaltered cell slightly above and to the right. Immediately above the hypertrophied nucleus a single oval cytoplasmic inclusion can be made out. It is in touch with the cell membrane and consequently very near the lumen of the duct. The original faint lilac color which it possessed is here indicated as a light gray. In Fig. 4 also essentially the same very light coloration of the inclusions (below the nucleus) is represented. Evidently thymonucleic acid is either absent in the inclusions or present in very small amounts.

Lastly, the Macallum test for potassium was employed with the modifications advised by Rohdenburg and Geiger.<sup>12</sup> All that can be said is that the black granular deposit which was obtained was

cent of acetic acid, and formalin in various concentrations. But it does not follow that they would be equally resistant to solvents applied to them in the fresh state, because Cowdry and Kitchen<sup>9</sup> found that the intranuclear inclusions characteristic of experimental yellow fever in monkeys, though equally resistant to fixatives containing acetic acid were quickly dissolved by the application of 0.05 per cent of acetic acid to the fresh cells.

Viewed in the fixed but unstained state the inclusions are not pigmented, neither are they doubly refractile when examined with crossed nicols. They are strongly basophilic and are thus easily distinguished tinctorially from the acidophilic intranuclear inclusions.

When frozen sections are made after formalin fixation the cytoplasmic inclusions can be tinged only very slightly with Sudan III, whereas neutral fat is strongly colored. Treatment with 2 per cent osmic acid for twenty-four hours results in a slight browning of the inclusions. When osmication is continued for several days a few small black granules make their appearance within the brown-colored inclusions.

Specific tests for mucin yielded rather inconclusive results. It was difficult to color the inclusions in the usual way with mucicarmine but by using a very concentrated sample of the stain they were induced to take a rather dense color which corresponded closely to "Nopal red" in Ridgway's standard color scale,<sup>10</sup> while all the other elements in the tissue were either unstained or but lightly tinged with the dye. This reaction appeared to be specific but should nevertheless be interpreted with caution.

In the first place, Dr. R. R. Bensley has kindly informed me, in a personal communication, that parts of dead cells can often be stained with mucicarmine irrespective of whether they do or do not contain mucus. There are two lines of evidence which indicate that the greatly enlarged duct cells with fully developed cytoplasmic and nuclear inclusions are dead. The first results from the use of methods designed to reveal mitochondria. Fig. 2 represents a cell after preservation in Regaud's fluid and coloration with anilin fuchsin and methyl green. In the original the mitochondria were colored crimson against a light green background. In the figure they are represented in shades of gray and may be recognized by their rod-like shape. It will be seen that, though mitochondria are present in abundance in the uninjured duct cells, they are conspicuous by their absence



invariably, seen. Sometimes and for no apparent reason they are more abundant than at other times. Cells fully charged with both nuclear and cytoplasmic inclusions may presumably persist for months, even years, without noticeable change. Though apparently dead for the reasons stated, they contain either in their interior or on their surfaces, active virus. In a few, however, the degeneration proceeds further as represented in the photomicrographs, Figs. 12, 13 and 14, and at the same time the cytoplasmic inclusions become less noticeable. Compare these figures with Figs. 10 and 11 which illustrate the height of the reaction in which both types of inclusion are very conspicuous.

This further degeneration of the injured cells is paralleled by non-specific changes in the neighboring duct cells, as seen in Fig. 12. By non-specific I mean that, by contrast, these other cells do not hypertrophy and do not develop inclusions of either kind. Their nuclei are lost by lysis and their cytoplasm fails to stain in the usual way and is replaced by chromophobic watery material. Examination of Fig. 12 shows that the lumen of the original duct is occupied by a very hypertrophied cell with a conspicuous intranuclear inclusion and that the adjacent cells to the left are further degenerated, in this way, than are those to the right. Accompanying such superposed non-specific lesions it is common to find a marked local infiltration consisting principally of lymphocytes and macrophages.

In Fig. 13 the degeneration is not quite so far advanced. The chief points of interest are absence of cytoplasmic inclusions, the extension of the surface of the cell and the failure of the cell to bulge into the lumen. In Fig. 14 the degeneration is so marked that the nuclear inclusion is hardly recognizable, the nuclear membrane itself is partly destroyed and no cytoplasmic inclusions can be made out.

While the vast majority of the injured cells remain in the condition illustrated in Figs. 10 and 11, some proceed further to the state represented in Fig. 14, and a few are definitely removed by desquamation into the lumina of the ducts. Such a cell is shown in Fig. 15. It contains only one cytoplasmic inclusion which is visible in the concavity of the nucleus.

The incidence of the two kinds of inclusions was also investigated in respect to the number of cell types in which they are produced. This was accomplished in three ways. First by the method of in-

limited to the cytoplasm of both the normal and injured cells. In the latter, however, as illustrated in Fig. 1, it was particularly abundant, especially in the distal region of the cytoplasm between the nucleus and the lumen of the duct, which area is known to contain the largest number of cytoplasmic inclusions.

### INCIDENCE

The examination of many guinea pigs of uncertain age was supplemented by the study of three guinea pigs of each of the following ages — 3, 5, 8, and 12 weeks. In this latter series the submaxillary glands were all fixed in Zenker's fluid and colored by Giemsa's stain.

No nuclear or cytoplasmic inclusions were found in the 3 weeks-old guinea pigs. Inclusions were observed only in one of the three guinea pigs killed at the age of 5 weeks, and all of them were nuclear. But all of the 8 and 12 weeks-old animals contained both nuclear and cytoplasmic inclusions. Approximately 10 per cent of the cells possessing intranuclear inclusions in the three guinea pigs of 8 weeks contained cytoplasmic ones, whereas about 50 per cent of them exhibited cytoplasmic inclusions in the three animals of 12 weeks. In support of these findings limited to so few animals it was observed in many instances that old-looking guinea pigs often contained both cytoplasmic and nuclear inclusions, whereas animals weighing less and presumably younger showed only nuclear inclusions.

It is evident that the cytoplasmic inclusions are of definitely restricted incidence in comparison with the nuclear ones. They appear only after the intranuclear inclusions are well formed and after the injured cells have undergone other profound changes, of which may be mentioned great hypertrophy and disappearance of mitochondria. In other words, it is likely that the cytoplasmic inclusions make their appearance at the time or after the death of the affected cells, whereas the intranuclear inclusions are first formed perhaps while the cells are still alive but at any rate before marked enlargement has set in. Figs. 6 and 7, for instance, illustrate intranuclear inclusions in relatively small cells which do not contain typical cytoplasmic inclusions. Even in old guinea pigs where the cellular hypertrophy is extreme, cytoplasmic inclusions are usually, but not

veloped in the duct cells of the parotid and of the mucous and serous portions of the submaxillary gland as well as in the serous acinous cells of the latter. They are never seen in the absence of intranuclear inclusions.

### DISCUSSION

What then is the status of these cytoplasmic inclusions? Until they have been seen in guinea pigs in injuries other than those caused by the submaxillary virus we may logically regard them in a provisional way as specific of its action. This specificity is however one-sided, for though the presence of cytoplasmic inclusions points to the presence of the virus, their absence does not mean that the virus is likewise absent; it indicates merely that the virus is weak. This statement is justified in view of Cole and Kuttner's experiments. Beginning with a saline emulsion of submaxillary glands containing many cytoplasmic and nuclear inclusions, it was a simple matter for them and for Scott and for me to create by intracerebral injection in guinea pigs a fatal meningitis characterized by an abundance of intranuclear inclusions without any cytoplasmic inclusions. But the second intracerebral passage very seldom if ever results in a distinct infection in spite of the large number of intranuclear inclusions in the material passed. Indeed Cole and Kuttner were only able to maintain the virus in a few serial transfers by alternating their sites of inoculation between testicle and brain and skin and brain. They do not state whether cytoplasmic inclusions occur in the testicle and skin. If they had been conspicuously present, reference would probably have been made to them. Summarizing the situation, it is safe to say that the virus has not yet been maintained by direct passage in unlimited series in any tissue except in the salivary glands in which the formation of the cytoplasmic inclusions is such a very conspicuous and apparently unavoidable feature of the cellular response.

It is possible therefore that the development of cytoplasmic inclusions is in some way concerned with the continuation of the virus in nature, or it may be that the cytoplasmic inclusions are merely incidental by-products of its action. Both hypotheses are rational in the light of our present scanty information. In terms of the latter hypothesis the conditioning factors in the formation of the cytoplasmic inclusions cannot as yet be given in detail. It may be a

tracerebral inoculation introduced for this virus by Cole and Kuttner. They say that "the most striking feature is the presence in the meningeal exudate of large numbers of cells, each of which contains an acidophilic mass within the nucleus. These cells resemble in all particulars the cells containing inclusion bodies which occur in herpes simplex and related conditions . . ."

Following the instructions given by these authors, I injected three young guinea pigs intracerebrally, and under ether anesthesia, with a 10 per cent emulsion in physiological salt solution of the submaxillary glands of several old guinea pigs. The animals were sacrificed seven days later and the intranuclear inclusions mentioned were found in the large mononuclear cells of the exudate, which however contained no trace of cytoplasmic inclusions.

The second method, likewise devised by Cole and Kuttner, was tried without success. It involved the removal, under ether anesthesia, of the submaxillary glands of three young guinea pigs. The wounds healed uneventfully and virus emulsion was injected subcutaneously at frequent intervals over a period of thirty days when the animals were sacrificed and the parotid glands prepared for study. The object was to induce the virus to act upon the parotids in the complete absence of the submaxillary glands, but no inclusions could be found in the parotids. Fortunately, however, Dr. Kuttner very kindly sent me a preparation of the parotid from such an experiment which showed beyond question both cytoplasmic and nuclear inclusions in the duct cells.

The third method of spreading virus action was that of Scott<sup>7</sup> and consisted of the ligation, under ether anesthesia, of the ducts of the submaxillary glands in a series of three young guinea pigs and immediate injection directly into each gland of 0.5 cc. of similar virus emulsion. After sixteen days the glands were removed and prepared for study. Typical cytoplasmic and nuclear inclusions were found in many duct cells as well as in a few of the cells of the serous acini, but nuclear inclusions unaccompanied by cytoplasmic ones were seen in addition in smooth muscle cells, fibroblasts, endothelial cells and large mononuclear cells.

Clearly, therefore, the formation of cytoplasmic inclusions is a much more restricted mode of response to the virus than that of the development of intranuclear inclusions when a variety of cell types is considered. In guinea pigs the cytoplasmic inclusions are de-

stantly produce both cytoplasmic and nuclear inclusions in all susceptible cells.

Whatever the significance of these cytoplasmic inclusions may be in the guinea pig, the fact that other similar cytoplasmic inclusions have been clearly described and illustrated by Wilson and Du Bois<sup>15</sup> in greatly enlarged duct cells of the submaxillary and parotid glands in a case of keratomalacia is of considerable interest because the inclusions afford another kind of cellular response which is common to guinea pigs and humans. A comparison of two photomicrographs by Wilson and Du Bois (Figs. 2 and 3<sup>15</sup>) with my photomicrographs (Figs. 10 and 11) shows that the similarity, not only in the cytoplasmic inclusions, but also in the nuclear ones and indeed in all other visible cellular attributes, amounts almost to identity. Study of the actual preparations side by side was made possible by the kindness of Dr. S. B. Wolbach in sending three specimens which form part of the material which will constitute the basis of a report from the department of pathology of the Harvard Medical School. The first specimen is of the submaxillary gland from the case described by Wilson and Du Bois (A-1009); the second of a parotid gland from a Children's Hospital autopsy (A-24-28) and the third of a lung from the case described by Goodpasture and Talbot.<sup>5</sup> *In the first two specimens I found cytoplasmic inclusions indistinguishable from those in guinea pigs. Rather less distinct cytoplasmic structures were observed in the third preparation of the lungs. But it is probable that identical cytoplasmic inclusions occurred in the lungs also, because Goodpasture and Talbot refer to the existence in them of cytoplasmic bodies similar to those reported in the guinea pig's submaxillary gland.*

It is probable that others before Goodpasture and Talbot, notably Jesionek and Kiolemenoglou<sup>16</sup> have observed the coexistence of nuclear and cytoplasmic inclusions in human salivary glands. The available evidence is, however, against the possibility that vitamine A deficiency is the causative factor in their production, which is suggested by their abundance in the case of keratomalacia, for the changes which result from experimental vitamine A deficiency have been carefully investigated by Wolbach and Howe,<sup>17</sup> and others, without bringing to light any inclusions, either nuclear or cytoplasmic. Far more likely is it that the inclusions in humans, as in guinea pigs, are caused by the action of a virus. The fact that the

question of the relative availability of material for the building up of the inclusions. Such material may not be present in sufficient amounts to form cytoplasmic inclusions in the large mononuclear, endothelial and other cells which habitually develop only intranuclear inclusions. Or again, it may be a case of the greater retention in the duct cells of material more rapidly removed from the other cells. Certain it is that the development of cytoplasmic inclusions in the duct cells of the submaxillary glands and their absence in other kinds of cells affected by the virus is but one of many fundamental differences in response. A second is the great hypertrophy of the duct cells and a third is their death and retention in the tissues without essential modification for months, perhaps for years, whereas the other kinds of cells are enlarged but little and are soon removed by phagocytic action. Persistence of the virus is provided for by cells which carry cytoplasmic inclusions in addition to nuclear ones.

Comparing now these cytoplasmic inclusions with other cytoplasmic inclusions described in the literature as caused by other kinds of viruses, we find no possibility of confusion. Like cytoplasmic inclusions in general as discussed by Cowdry<sup>13</sup> they are rather resistant to solvents contained in fixatives so that no special technique is required for their demonstration. They are basophilic like some of the inclusions in infectious myxomatosis of rabbits and trachoma bodies, but here the similarity ends owing to their distinctive morphology. Each inclusion is made up of many fine particles, as is the case in birdpox; but in the submaxillary disease the lipoidal component is wanting. From typical large Negri bodies they are easily distinguished by the absence of any acidophilic material. Finally it may be said that the submaxillary cytoplasmic inclusions are not made up of the heterogeneous material which is typical of Guarnieri bodies; but like the Guarnieri bodies (Cowdry,<sup>14</sup> Fig. 37) they are of such firm consistency that they indent the nuclei when in contact with them.

The submaxillary virus is rightly grouped with viruses like those of herpes, chickenpox, Borna disease and other conditions which are characterized only by the formation of intranuclear inclusions. The cytoplasmic inclusions described in this paper are not so inseparable from the action of the virus as to justify the reclassification of the virus with those of variola and paravaccinia which con-

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same duct cells of the same glands are affected, and, further that they are altered, as far as we can tell in precisely the same way, indicates that the guinea pig and human viruses are very similar, if they are not one and the same. We have other instances of individual viruses which can be induced to bring about similar cellular modifications in the cells of humans and guinea pigs (herpes, vaccinia, rabies, etc.) but none are recognized which do so naturally in a spontaneous way.

## RESULTS

The cytoplasmic inclusions which are developed in the duct cells of the submaxillary glands of guinea pigs as a result of the action of the submaxillary virus are formed later than are the nuclear inclusions. They are also more restricted in distribution, having been found only in the ducts of the mucous and serous portions of the submaxillary, occasionally in the acini and in the ducts of the parotid; whereas the intranuclear inclusions may in addition be produced in endothelial cells, fibroblasts, smooth muscle cells and mononuclear leucocytes. The cytoplasmic inclusions are spherical or oval structures which vary in size from a fraction of a micron up to 6 to 8 microns in diameter. In the fully developed state the average inclusion is about 3 microns in its long axis. The inclusions are made up of many much smaller individual particles which are densely packed together. Like the cytoplasmic inclusions in other virus diseases, these inclusions are characterized by their relative insolubility in ordinary fixatives. They are basophilic in reaction and do not contain fat or lipoid in detectable amounts. They are, moreover, indistinguishable from certain cytoplasmic inclusions of rare occurrence in human submaxillary glands.

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FIG. 8. An inclusion-laden cell of an intercalated duct from the submaxillary gland of an adult guinea pig. Note the particularly large and well developed cytoplasmic inclusions. The affected cell has completely plugged the intercalated duct. Giemsa's stain.  $\times 2000$ .

FIG. 9. An affected cell found in the duct of the minor sublingual gland of an adult guinea pig. A cytoplasmic inclusion may be seen adjacent to the nucleus. Hematoxylin and eosin.  $\times 1500$ .

## DESCRIPTION OF PLATES

## PLATE 62

All of these figures were originally drawn in colors with the aid of a camera lucida. They have been reduced to black and white by photography.

FIG. 1. An inclusion-laden cell from the secretory duct of the submaxillary gland of an adult guinea pig. The Macallum test for potassium revealed deposits localized in areas which correspond topographically to those occupied by the cytoplasmic inclusions (compare with Fig. 8). Potassium deposits are black in the figure. Two adjacent normal cells are represented above the cell containing the nuclear inclusion.  $\times 2000$ .

FIG. 2. An affected cell from the submaxillary gland of an adult guinea pig. The gland was fixed in Regaud's fluid and stained with anilin acid fuchsin and methyl green to reveal mitochondria, which appear as black, straight and slightly curved rods. The normal secretory duct cells have abundant mitochondria, whereas the inclusion-laden cell shows a complete absence of these cytoplasmic bodies.  $\times 2000$ .

FIGS. 3 and 4. Affected cells from the submaxillary gland of an adult guinea pig. The Feulgen reaction for thymonucleic acid has been applied to these cells. In Fig. 3 the intranuclear inclusion stains but faintly, which is in contrast to the strongly positive particles of chromatin adhering to it. A cytoplasmic inclusion is shown slightly tinged in the upper portion of the cell. In Fig. 4 the nuclear inclusion is but faintly tinged with the dye as is the group of cytoplasmic inclusions. The nuclei of normal adjacent secretory duct cells give a strongly positive reaction as can be seen in the figures.  $\times 2000$ .

FIGS. 5 and 6. Inclusion-bearing acinar cells of the submaxillary gland of an adult guinea pig. The degree of nuclear hypertrophy is not so marked as is that of the secretory duct cells. In Fig. 5 the cell shows a strongly basophilic reaction of the cytoplasm but no visible cytoplasmic inclusions. Giemsa's stain.  $\times 1500$ .

FIG. 7. A binucleate fibroblast, each nucleus of which contains an inclusion body. There are no cytoplasmic inclusions present. Taken from a section of the submaxillary gland of an adult guinea pig. Giemsa's stain.  $\times 2000$ .

## PLATE 63

All of these figures are photomicrographs taken by Mr. Louis Schmidt of the Rockefeller Institute at a magnification of 2000 diameters from preparations fixed in Zenker's fluid and colored by Giemsa's stain.

FIG. 10. Shows two cells containing both cytoplasmic and nuclear inclusions on opposite sides of a secretory duct.

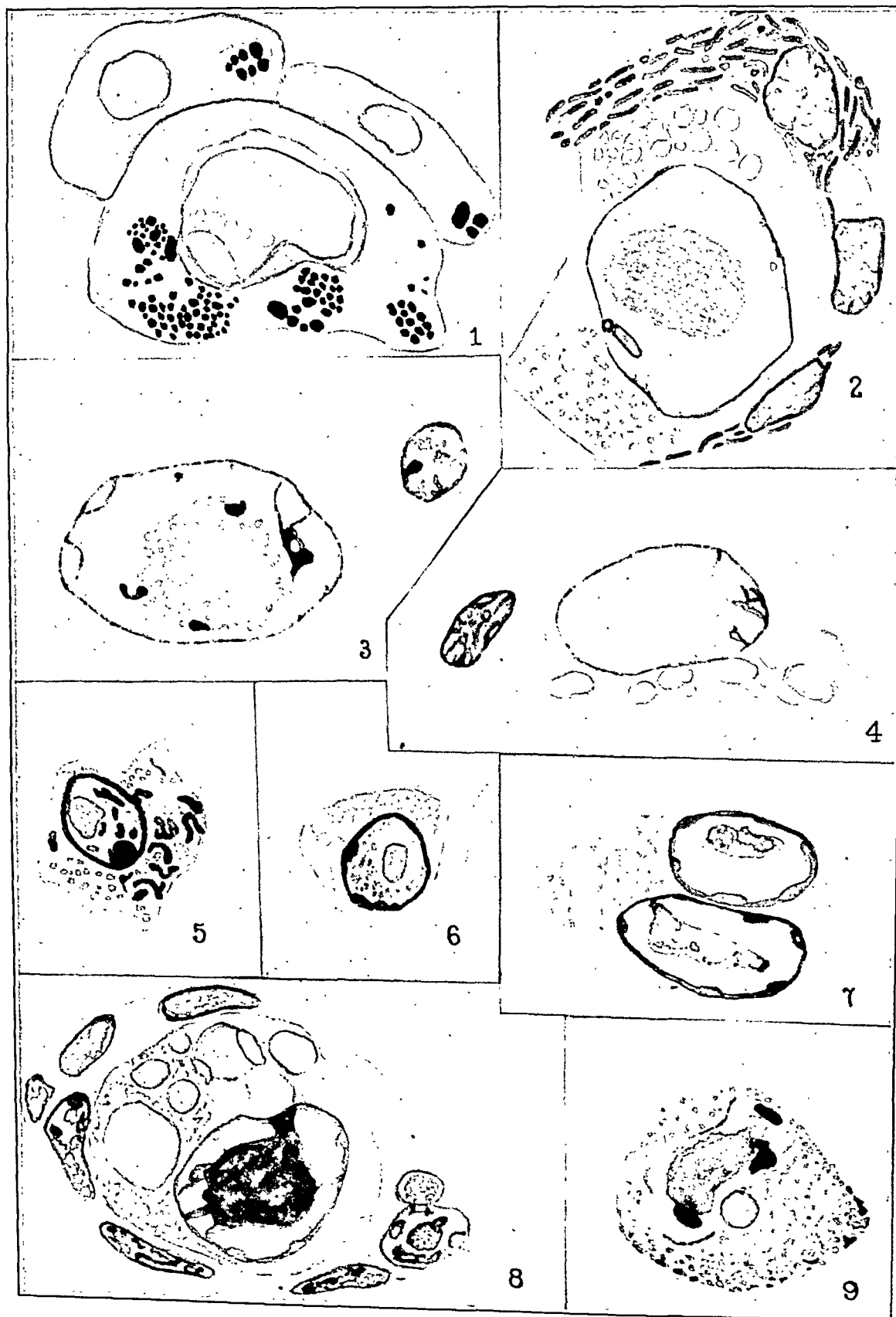
FIG. 11. Represents a single cell laden with both types of inclusions. The cytoplasmic inclusions exercise pressure on the nuclear membrane and indent it.

FIG. 12. Shows a duct almost completely blocked with a greatly hypertrophied cell containing a large intranuclear inclusion. Cytoplasmic inclusions are not visible. The unenlarged duct cells are severely injured, particularly to the left.

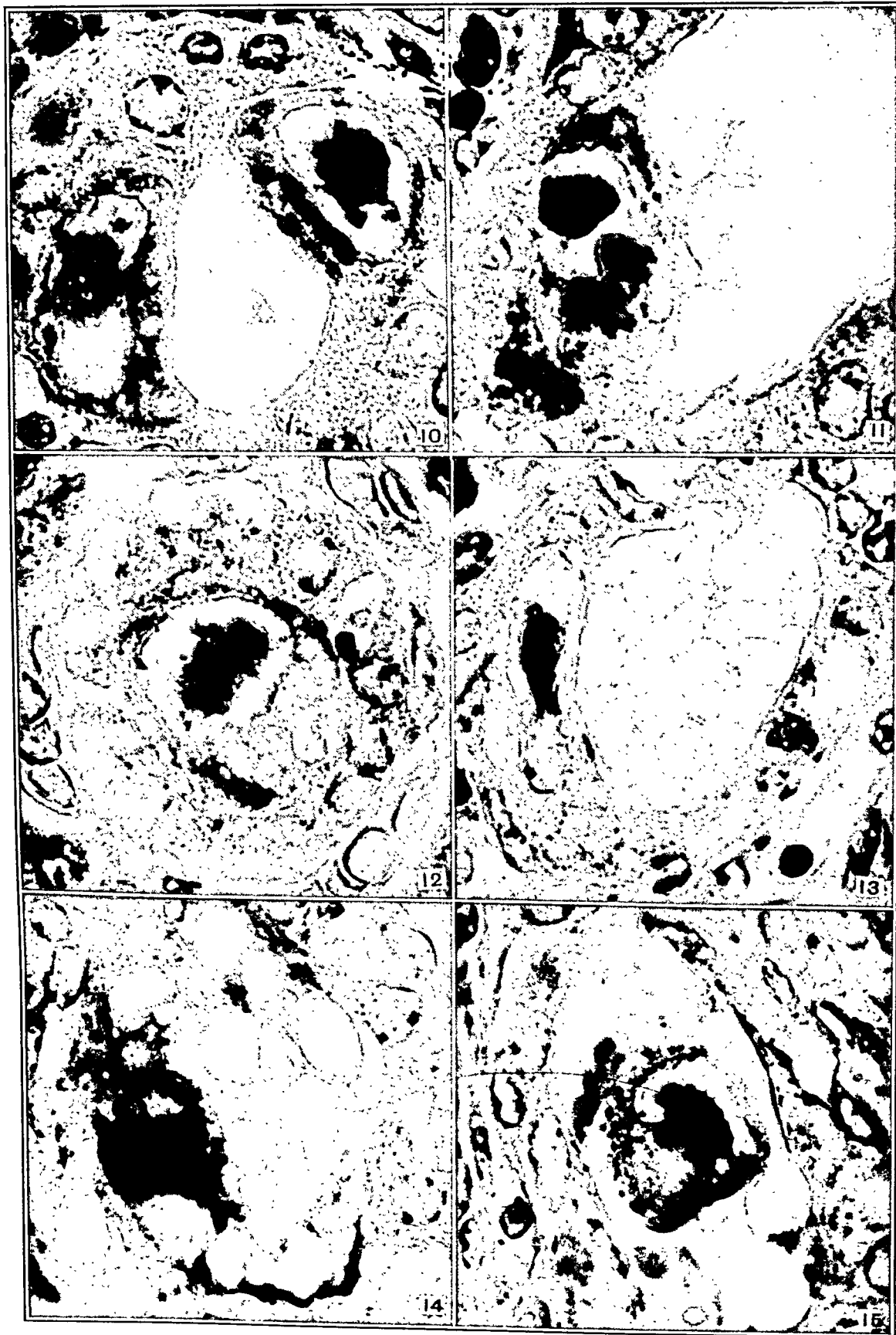
FIG. 13. Represents likewise an enlarged cell possessed of a distinct nuclear inclusion but no cytoplasmic ones. The condition is unusual because the cell does not bulge into the lumen.

FIG. 14. Illustrates a very degenerated cell bordering the lumen in which neither nuclear nor cytoplasmic inclusions can be clearly distinguished.

FIG. 15. Shows an inclusion-bearing cell which has been cast off into the lumen. A single spherical cytoplasmic inclusion can be seen in the concavity of the nucleus.







largely from dealers in Maryland and Pennsylvania. All animals were examined on admission and were found to be clinically negative as regards thyroid enlargement.

All of the animals used in the experiments described below were of known stock and age. The animals in each experimental and control group were of the same age and the groups were so arranged that the number of males and females was the same in each group. Because of this uniformity of experimental material, absolute thyroid gland weights are given throughout, instead of thyroid gland, body weight ratios.

### EXPERIMENTAL

*Experiment No. 1:* In view of the extensive work of McCarrison<sup>2</sup> and others on the relationship of food contamination to the etiology of goiter, an attempt was made to determine whether or not this factor played any part in the present epidemic. Accordingly, a special system of caging was instituted with the idea of removing, as far as possible, the factor of fecal contamination of the food. These cages were of metal and measured 61 by 46 by 31 cm. The top and front were of wire mesh. The bottom consisted of a removable metal pan over which was placed a grid of coarse wire mesh. Through this grid the urine and feces dropped to the pan below. The pans were so arranged that they drained into a central drainage system. In this way, the bottom of the cage, on which the animal rests, was always clean and dry. At frequent intervals the rabbits were removed and the interior of the cages was washed by means of a hose. Food was placed in glazed earthenware cups, 3 inches high. These cages were placed in a well ventilated, well lighted part of the animal room. By this manner of caging, fecal and urinary contamination of the food was almost entirely eliminated.

Five litters, consisting of thirty-two rabbits of approximately the same age, were used for this experiment. Sixteen rabbits were placed in the special cages described above. The remainder were housed in standard cages and were intended to serve as a control series. The litters were divided into approximately equal parts. The diet in both instances was identical, the standard laboratory diet being used. The mortality from intercurrent infections was high during the course of this experiment. At the end of approxi-

## STUDIES IN THE ETIOLOGY OF SIMPLE GOITER \*

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### INTRODUCTION

In a previous communication<sup>1</sup> we have reported the occurrence of goiter in a colony of rabbits maintained upon a diet consisting almost exclusively of cabbage. The condition was identified as simple or endemic goiter. The microscopic picture usually presented was that of the typical *struma diffusa parenchymatosa*. In view of the multiplicity of etiological factors with which simple goiter is commonly accredited, it was considered advisable to attempt to rule out some of these as contributory factors in the present epidemic. This communication deals with a series of experiments which were carried out in an effort to determine whether or not the cabbage diet was the sole etiological factor in this instance and, if not, what rôle certain other factors played in the production of goiter. Since goiter occurred with great regularity in all stock rabbits kept under standard conditions in our laboratory, it is considered advisable to describe these standard conditions briefly.

The rabbits were housed in quarters on the eighth floor of the dispensary building of the Johns Hopkins Hospital. The room is well ventilated and well lighted, having a southern and eastern exposure. The cages are supported on metal racks, three tiers high with ample ventilation space between individual cages. The standard cage is of metal and measures 45 by 30 by 30 cm. The front and top are made of wire mesh. The bottom of the cage is covered with shavings which are changed once or twice weekly. The standard diet consists of a daily ration of approximately 250 gm. of cabbage and a weekly ration of approximately 20 gm. of hay and 50 gm. of oats. The cabbage was procured from various regions in the Eastern United States, ranging between Florida and New York state with the season. Water was not given on the standard diet. No special breed of rabbits was used, the stock being procured

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mately three hundred and forty days, the animals still living were sacrificed and autopsied. Table I shows the results of the above experiment.

It will be observed from an inspection of Table I, that goiter developed in the animals kept in the special cages where fecal and urinary contamination was reduced to a minimum (Group 1), as readily as in the animals kept in cages where there was abundant opportunity for fecal and urinary contamination. The average weight of the thyroid glands at the termination of the experiment was 2.83 gm. for the group housed in the special cages, and 2.70 gm. for those housed under standard laboratory conditions.

*Experiment No. 2:* Since water has been associated with the etiology of simple goiter by all peoples from the remotest times<sup>3</sup> it was considered advisable to determine whether or not the absence of water was a factor in the present epidemic, and at the same time to find out if the traces of iodine contained in Baltimore city tap water \* were sufficient to protect the animals against a powerful goitrogenic influence such as we were dealing with in this instance. Thirty rabbits of approximately the same age and from the same source were divided into three equal groups. All were placed on the standard cabbage diet and were housed under the standard laboratory conditions. Group 1 was constantly supplied with freshly distilled water in glazed earthenware containers. Group 2 was similarly supplied with Baltimore city tap water. Group 3 served as a control series and was given no water to drink. At the end of one hundred and twenty-five days all the animals were sacrificed and autopsied. Table II shows the results of this experiment.

This experiment was terminated at an earlier date than in the case of the preceding one, hence the thyroid weights were smaller. Previous experience had shown that time is an important factor in the development of the larger goiters in rabbits maintained on the goiter-producing diet. The table shows that the average weight of the thyroid gland in Group 1 (which received distilled water) was 0.57 gm., while the average weight in Group 2 (which received tap water) was 0.53 gm. In the control series the average weight of the thyroid gland was 0.67 gm. Microscopic sections of the thyroid glands of all animals sacrificed at the termination of the experiment showed varying degrees of hyperplasia.

\* Two parts per billion, as estimated in laboratories of City Water Department.

TABLE I  
Incidence of Goiter in Rabbits Maintained in Two Types of Cages

Group I					Group II				
Special Cages					Standard Cages				
Rabbit number	Weight of thyroid gm.	Days in laboratory	Cause of death	Microscopic appearance of thyroid	Rabbit number	Weight of thyroid gm.	Days in laboratory	Cause of death	Microscopic appearance of thyroid
2111	2.5	345	sacrificed	marked hyperplasia	2107	1.2	345	sacrificed	marked hyperplasia
2112	14.0	345	"	"	2108	1.3	345	"	"
2113	2.8	345	"	"	2109	8.4	345	"	"
2114	2.8	345	"	"	2110	3.5	345	"	"
2136	0.8	330	"	hyperplasia	2140	2.5	345	"	"
2188	0.5	325	"	"	2190	1.2	345	"	"
2189	0.9	325	"	"	2191	0.8	345	"	"
2182	0.4	325	"	"	2185	2.7	345	"	"
2195	1.8	325	"	"	2139	3.8	276	pneumonia	"
2196	1.2	270	pneumonia	"	2192	0.65	258	"	hyperplasia
2138	0.3	97	diarrhea	slight hyperplasia	2137	0.6	172	"	"
2135	0.4	87	caseous abscess	"	2197	0.6	141	"	"
2183	0.3	75	diarrhea	"	2199	0.4	88	diarrhea	slight hyperplasia
2187	0.3	72	"	"	2198	0.2	55	"	normal
2184	0.1	60	"	normal	2186	0.7	52	"	hyperplasia
2194	0.2	50	pneumonia	"					

*Experiment No. 3:* An attempt was made to demonstrate the effect of iodine prophylaxis against an active goitrogenic agent such as the cabbage diet. Four litters consisting of sixteen rabbits, were divided into two equal groups containing representatives of each litter. These animals were all placed in standard cages and maintained on the standard cabbage diet. Group A received 7.5 mg. of iodine per week by mouth. Group B served as a control series and received no iodine. At the end of approximately four hundred days, all of the surviving animals of Group B had visible and readily palpable goiters, while in none of the Group A animals could the thyroid gland be felt at this period. The rabbits were then sacrificed and autopsied. Table III shows the results of this experiment.

Here it can be seen that all of the animals in Group A had thyroid glands which were within the normal weight range (average 0.25 gm.), while all those in Group B had moderate-sized goiters, the average thyroid gland weighing 2.3 gm.

During the course of the above experiments, several striking aspects of the disease manifested themselves. There was considerable individual susceptibility on the part of certain animals toward the goitrogenic agent. Thus, in Table III, Group B, one sees that rabbit No. 2029 had a thyroid gland which weighed 7.3 gm. while numbers 2028, 2030 and 2031, which were from the same litter and were kept under identical conditions, had thyroid glands weighing 1.8, 1.4 and 1.4 gm. respectively. This same variation may be noted in many of the other tables. A marked seasonal variation was also noted. In animals brought into the laboratory in the late autumn or winter, clinically detectable goiters developed much more quickly than in those procured in the spring or summer months. Similarly, some of the large goiters tended to decrease in size slightly during the spring and summer. Throughout the present epidemic no greater incidence in females than in males has been noted. There is suggestive evidence that young rabbits are more susceptible to the goitrogenic influence than adult ones. No variation in the incidence of goiter has been noted among the different breeds of rabbits.

TABLE II  
*Effect of Adding Water to the Diet on the Incidence of Goiter in Rabbits*

Group 1						Group 3						Group 2					
Distilled Water Series						Control Series						Tap Water Series					
Rabbit number	Weight of thyroid	Days in laboratory	Cause of death	Microscopic appearance of thyroid	Rabbit number	Weight of thyroid	Days in laboratory	Cause of death	Microscopic appearance of thyroid	Rabbit number	Weight of thyroid	Days in laboratory	Cause of death	Microscopic appearance of thyroid	Rabbit number	Weight of thyroid	Days in laboratory
2397	gms. 0.80	125	sacrificed	hyperplasia	2400	gms. 0.70	125	sacrificed	hyperplasia	2390	gms. 0.25	125	sacrificed	slight hyperplasia	2390	gms. 0.25	125
2380	0.80	125	"	"	2401	0.60	125	"	"	2391	0.30	125	"	"	2391	0.30	125
2382	0.40	125	"	"	2402	0.90	125	"	"	2392	0.55	125	"	hyperplasia	2392	0.55	125
2383	0.30	125	"	slight hyperplasia	2404	0.50	125	"	"	2393	0.55	125	"	"	2393	0.55	125
2384	0.40	125	"	hyperplasia	2405	0.50	125	"	"	2394	0.65	125	"	"	2394	0.65	125
2385	1.40	125	"	"	2406	0.65	125	"	"	2395	1.00	125	"	"	2395	1.00	125
2386	0.45	125	"	"	2407	0.60	125	"	"	2396	0.30	125	"	slight hyperplasia	2396	0.30	125
2387	0.55	125	"	"	2408	0.50	125	"	"	2397	0.60	125	"	hyperplasia	2397	0.60	125
2389	0.50	125	"	"	2410	1.40	125	"	"	2398	0.65	125	"	"	2398	0.65	125
2388	0.10	20	pneumonia	normal	2409	0.40	34	pneumonia	slight hyperplasia	2399	0.45	125	"	"	2399	0.45	125

## DISCUSSION

The experiments outlined above appear to indicate that the major etiological factor in the epidemic of simple goiter under investigation, is a nutritional one. The cabbage diet, on which the animals were maintained, apparently exerted a very active goitrogenic influence.

These experiments are not in accord with the extensive investigations of McCarrison,<sup>2</sup> in which he attempted to show that unhygienic surroundings and fecal contamination of food were important causative factors in endemic and experimental goiter. In Experiment No. 1 there was no greater tendency to thyroid hyperplasia when the animals were kept under conditions permitting easy contamination of food than when they were maintained in the special cages under the best possible conditions.

Drinking water has been associated with the etiology of simple goiter by countless writers on the subject.<sup>3</sup> In this instance, since goiter occurred when the animals were given no water to drink, one might conceive of the goitrogenic factor as being due to the absence, rather than the presence of, some water-borne substance. Experiment No. 2, however, shows that the addition of either tap or distilled water to the diet has little or no effect on the goitrogenic factor. The slightly lower level of the average thyroid gland weights of the groups receiving water (0.57 and 0.53), as compared with those of the control group (0.67), is well within the range of accuracy of such a method of observation. The amount of iodine or other inorganic substances in the tap water of Baltimore city apparently is not sufficient to cause any detectable effect different from that given by distilled water.

As Marine has repeatedly shown, iodine in sufficient quantities will protect against all known goitrogenic agents. Experiment No. 3 shows clearly that 7.5 mg. of iodine per week was sufficient to afford the animals complete protection for a period of over one year. It is probable that much smaller amounts would have sufficed. The traces of iodine contained in the drinking water exerted no demonstrable effect. The exact amount necessary to protect lies somewhere between those two extremes.

The experiments outlined above appear to rule out fecal and urinary contamination of food, and deficiency in water-borne sub-

TABLE III  
Showing the Effect of the Addition of 7.5 mg. of Iodine Per Week to the Diet on the Incidence of Goiter in Rabbits

Group A					Group B				
Standard Diet plus 7.5 mg. Iodine per week					Standard Diet				
Rabbit number	Weight of thyroid	Days in laboratory	Cause of death	Microscopic appearance of thyroid	Rabbit number	Weight of thyroid	Days in laboratory	Cause of death	Microscopic appearance of thyroid
	gm.					gm.			
1994	0.30	420	sacrificed	colloid increased, no hyperplasia	1998	1.75	420	sacrificed	extreme hyperplasia
1996	0.25	420	"	"	2000	2.0	420	"	"
1997	0.30	420	"	"	2001	1.6	420	"	"
2033	0.20	400	"	"	2028	1.8	400	"	"
2035	0.30	400	"	"	2029	7.3	400	"	"
1995	0.20	200	pneumonia,	"	2030	1.4	400	"	"
2032	0.30	130	"	"	2031	1.4	400	"	"
2034	0.10	25	none found	"	1999	1.1	225	pneumonia	"

more active in winter than in the summer months. This goiter-producing factor appears to be a nutritional one and may act through the oxidation-reduction systems of the body.

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stances as accessory factors in the etiology of the present epidemic of simple goiter. It would appear that the cabbage diet on which the animals were maintained contains some powerful goitrogenic agent. Further, our experience has been that there is considerable seasonal variation in the incidence of goiter in these animals. This could be due to seasonal increase in the goitrogenic factor or to variation in the animals' susceptibility at certain time periods. The extreme variation in susceptibility of individual animals from the same litter is of great interest. One has to presuppose a so-called "constitutional factor" here which may conceivably operate through other endocrine glands and exert a protective influence, in certain instances, against the goitrogenic agent.

Since the above experiments were concluded, Marine, Baumann and Cipra<sup>4</sup> have added considerable light to our knowledge of cabbage as a goitrogenic agent. They have confirmed our observation that a cabbage diet will produce thyroid hyperplasia in rabbits, and have further shown that other members of the Brassica group will also produce goiter. They have suggested that cabbage acts as a goitrogenic agent by depleting the thyroxin store in the thyroid, thus producing a relative iodine insufficiency in the animal, which in turn causes hyperplasia of the thyroid gland. Marine's suggestion that this is brought about by some powerful reducing substance contained in the cabbage seems probable, and offers a possible explanation of many of the hitherto seemingly unrelated etiological factors of simple goiter.

#### SUMMARY

Further investigations into the etiological factors involved in an epidemic of simple goiter in rabbits are reported. A diet which consists almost exclusively of cabbage appears to be the major etiological factor. Fecal and urinary contamination of food seemingly play no rôle in the present epidemic. The addition of water (either tap or distilled) to the diet exerts no appreciable protective influence against the goitrogenic agent. Iodine, administered orally in quantities of 7.5 mg. per week will completely protect the animal against the goiter-producing factor. There is no evidence that the minute traces of iodine contained in Baltimore city tap water exert any detectable protective influence. The goitrogenic agent is much



interpreted this as chronic parenchymatous nephritis. Kleine<sup>8</sup> in a recent article described a large white kidney with narrow obscure cortex in a multiple myeloma. He found a marked nephrosis with lamellated casts in the intact tubules and syncytial giant cells at their periphery. These casts were digested by trypsin, were insoluble in alcohol, water and weak acid, and dissolved with antiformin. He interpreted the lesions as parenchymatous and interstitial nephritis. Bannick and Greene<sup>9</sup> reported in considerable detail the clinical histories of thirteen instances of Bence-Jones albumosuria, associated with renal insufficiency. There was marked secondary anemia, non-protein nitrogen retention, low specific gravity of the urine with no hematuria and usually without hypertension or retinitis. Although they did not report any autopsy findings, they believed the condition was associated with extensive tubular destruction with subsequent fibrosis or pyelonephritis. Arteriosclerosis and hypertension occurred in some instances.

#### CASE I.

*Clinical History:* H. A., age 43 years, was admitted to the medical service of Montefiore Hospital on March 19, 1929 with the complaints of pain in the chest, cough and loss of weight of six months duration. The present illness began six months ago with knife-like pains in the left lower chest radiating to the back and neck. The pain increased on exertion. During the last few weeks he had had weakness, dizziness on exertion, anorexia, constipation and, during the last four days, vomiting. The patient was well built. His skin had a waxy pallor. Tender areas were felt over the sixth and seventh ribs on the anterior chest wall. There was a short systolic apical murmur with no enlargement of the heart. The blood pressure was 130/80. Examination of the lungs, abdomen and extremities revealed no abnormalities. Rectal examination showed a small prostate free of nodules.

*Laboratory Data:* Blood Wassermann was negative. Urea nitrogen 64, creatinin 5.5, calcium 14.6, phosphorus 6.2, basal metabolism rate minus 25 per cent. Examination of blood on March 21st: red blood cells 2,560,000, hemoglobin 48 per cent (Dare), white blood cells 6,400, polymorphonuclears 62 per cent. May 2nd: red blood cells 1,150,000, hemoglobin 15 per cent.

The urine contained large amounts of albumin and of Bence-Jones albumose. The sediment contained granular casts and white blood cells. Concentration (dry) showed a specific gravity of 1011, 1010, 1011. Roentgen-ray examination revealed numerous areas of rarefaction in the skull, ribs, seventh dorsal vertebra and pelvis.

*Clinical Diagnosis:* Multiple myeloma with Bence-Jones proteinuria and nephrosis. The patient received a series of five X-ray treatments. Two months after admission, hemorrhages from the gums and in the eye-grounds were noted. The patient became progressively weaker, developed twitching movements, uremic manifestations, and died.

## NEPHROSIS IN MULTIPLE MYELOMA \*

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Although the association of pathological changes in the kidney with multiple myeloma has long been recognized, the exact nature of these changes has not been sufficiently emphasized. In the two instances of multiple myeloma reported in the present communication, a severe nephrosis complicated the disease.

In a careful survey of the literature by Wallgren,<sup>1</sup> and Geschickter and Copeland<sup>2</sup> an attempt was made to study the kidney lesions. Of the vast number of communications on the subject of multiple myeloma, descriptions of the morphology of the kidney were found in less than 20 per cent of the instances. In most of these the descriptions were inadequate. Since the more recent knowledge of the nephroses was entirely unknown to the earlier pathologists, it is reasonable to assume that its appearance was often overlooked or certainly misinterpreted. From the description in the case reports of Scheele and Herxheimer,<sup>3</sup> Austin<sup>4</sup> and Beck and McCleary,<sup>5</sup> a marked destruction of tubules was noted with secondary interstitial changes. Thannhauser and Krauss<sup>6</sup> in 1921 report in considerable detail changes in the kidney indicative of a nephrosis. The kidneys were small and white. Microscopically they showed a diffuse, severe degenerative change of the entire tubular system with very little involvement of the glomeruli. The tubules were replaced by a richly cellular connective tissue. In addition there were small foci of lymphocytes. In some areas tubules were recognizable but showed degenerative changes. Peculiar lamellated casts were seen in the lumina of the intact tubules. On the outer layer were occasional foreign body giant cells. Thannhauser and Krauss believe these structures are concretions of serum protein and Bence-Jones albumose. Clinically, the patient had had evidence of nephrosis. This is the first instance of a pure nephrosis in multiple myeloma noted in the literature.

McConnell<sup>7</sup> in a report of an instance of multiple myeloma describes extensive tubular destruction in the kidneys with hyalinized glomeruli as well as calcification in the tubules. He erroneously

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*Bones:* The ribs were extremely brittle, moderate pressure from without causing fracture of most of them. In places they presented fusiform swellings. In these areas, the cortex was reduced to a thin shell and was readily broken. The underlying marrow was grayish red in color, somewhat firmer than normal and rubbery in consistency. The medullary tissue in the lumbar vertebrae was irregularly grayish red in color and presented moderately firm grayish areas similar to those in the ribs. The medullary tissue in the shaft of the right femur likewise contained firm rubbery grayish pink tissue. The cortex, however, was of the usual thickness.

### MICROSCOPIC EXAMINATION

*Heart:* In the heart there are areas of atrophy. Many of the fibers contain fine fat droplets which stand out readily when stained with Sudan III.

*Liver:* There is slight central fibrosis of the lobules of the liver and brownish pigmentation about the nuclei of the cells. Many of the sinusoids contain large vesicular cells similar to those seen in the section of bone marrow.

*Spleen:* The follicles of the spleen are few and small. There are large amounts of blood pigment throughout the pulp, some free in the interstitial tissue and some in the endothelial cells. In the sinuses of the pulp are seen many cells of the type described in the bone marrow. A moderate amount of congestion is present.

*Kidneys:* The glomeruli of the kidneys are for the most part intact. Some, however, show thickening and dilatation of the capillary loops and a diminution in the number of endothelial elements. In some places they are somewhat smaller than normal. The convoluted and collecting tubules show considerable change. In the cortex the tubules are dilated and filled with light pink-staining material (Fig. 1). In the medulla the tubules show extensive degenerative changes. In many places the epithelium is desquamated. The nuclear elements are faintly staining. The interstitial tissue is markedly increased. There are areas where the tubular elements are completely destroyed and replaced by a few scattered cells, apparently remnants of tubules and hyalinized edematous connective tissue (Fig. 3). Everywhere the tubules that are intact contain pink-staining material. In a few areas in the medulla the tubules

## AUTOPSY REPORT

*Anatomical Diagnoses:* The anatomical diagnoses were: Multiple myeloma involving ribs, vertebrae, femora, ossa innominata, liver and spleen; chronic nephrosis with contraction of the kidneys; fatty metamorphosis of the heart; parenchymatous degeneration of the viscera; acute splenic tumor; edema of the lungs; localized fibrinous pericarditis, and subendocardial ecchymosis.

*Skin:* The skin had a brownish tint and there was very marked pallor of the mucous membranes.

*Heart:* The heart weighed 350 gm. There was a moderate dilatation of the chambers. The epicardial surface of the right auricle presented a large area of ecchymosis. The musculature was flabby, and a pale brownish yellow. There was tigering of the papillary muscles on both sides. The valves showed no abnormalities except for several fine filiform gray vegetations on the posterior aortic cusp. There was diffuse ecchymosis beneath the endocardium in the right auricle. The coronaries were soft and patent throughout and free of atheromatous change.

*Lungs:* The lungs were edematous. The pleura over the right lower lobe presented a dull fibrinous exudate.

*Liver:* The liver weighed 1600 gm. It was soft and flabby in consistency. Cut surface was brownish in color and the lobules were indistinct. Scattered throughout the liver was seen an occasional pin-head sized, grayish dot.

*Spleen:* The spleen weighed 110 gm. It was very soft and flabby. Its cut surface was brick red in color and there were numerous small petechial hemorrhages within the pulp. The corpuscles were not clearly visible. The pulp was mushy in consistency and scraped away readily on the knife.

*Kidneys:* The kidneys together weighed 220 gm. and each measured 10 by 6 by 3 cm. The capsule stripped readily. The cortical surface was extremely pale and had a yellowish color. The stellate veins were prominent. The organs were extremely soft and flabby and the cut surface was very pale. The cortex was everywhere narrow and varied from 2 to 4 mm. in thickness. The medulla measured 15 mm. in thickness and was differentiated from the cortex with difficulty. The cortical markings were almost completely obscured. Pelves and ureters showed no abnormalities.

at the apex, but the heart was not enlarged. The blood pressure was 135/75. There was kyphosis of the dorsal, and part of the lumbar spine with tenderness over the lower vertebrae. X-ray of the skull showed numerous punched out areas of rarefaction. Areas of rarefaction were found in lumbar vertebrae, pelvic bones, upper halves of the femora, the ribs, clavicles, scapulae and upper halves of the humera. The red blood cell count was 1,220,000, hemoglobin 20 per cent, white blood cell 7,800. Blood urea 52.2, creatinin 5.1. Wassermann negative. The urine had a specific gravity of 1012, and contained large quantities of albumin but no Bence-Jones albumose. The sediment contained some pus cells but no casts. Five days after admission the patient died with signs of a terminal pneumonia.

### AUTOPSY REPORT

*Anatomical Diagnoses:* The anatomical diagnoses were: Multiple myeloma involving the ribs, spine and innominate bones; chronic nephrosis with contraction of the kidneys; fatty metamorphosis of the heart; cholelithiasis; prostatic hypertrophy; dilatation and hypertrophy of the bladder; polyps of the colon.

The body was that of an emaciated elderly man with a peculiar waxy pallor of the skin and mucous membranes. The upper part of the chest was flattened. The lower part bulged anteriorly and laterally. The ribs were very brittle and fractured readily on pressure.

*Heart:* The heart weighed 230 gm. The myocardium was pale and flabby and small pin-head yellow patches were scattered throughout. The chambers were of normal size. The endocardium and valves showed no abnormalities.

*Lungs:* The pleura were smooth and glistening except for fibrous tags at the bases. The upper and middle lobes were soft and crepitant. Both lower lobes were firm, airless, and on section had a dark red meaty appearance. From the lower lobes, dirty red fluid could be expressed from the cut surface. Bronchial mucosa and vessels showed no abnormalities.

*Gall Bladder:* The gall bladder was thin-walled and contained numerous pyramidal facettted black and yellow stones. Stones were found in the hepatic and cystic ducts but the common duct was patent.

*Spleen:* The spleen weighed 210 gm. and measured 14 by 7 by 3 cm. It was large and soft and on section rust brown in color. The corpuscles were not seen. The trabeculae were thin and delicate. Pulp scraped with difficulty. No tumor nodules were present.

contain calcified masses (Fig. 2), and occasionally one finds in the lumen of some of the tubules some lamellated pink-staining material resembling concretions. The interstitial tissue throughout appears to be very cellular. On closer observation the cellular appearance is due to an increase in connective tissue elements although in some areas there are collections of lymphocytes. There is evidence of slight arteriosclerosis with thickening of the smaller vessels and connective tissue replacement of an occasional glomerulus. The chief lesion seems to have affected the tubular elements of the kidney and apparently is a very severe nephrosis.

Fat stain shows a considerable number of fine fat droplets in the epithelium of the convoluted and collecting tubules.

*Bone Marrow:* The bone marrow from the femur shows extensive hemorrhage. The normal bone marrow tissue is replaced by large masses of densely packed cells. These cells are round, polygonal and oval in shape. The cytoplasm for the most part is indistinct. The nuclei are large, round and vesicular. Some are slightly compressed and spindle-shaped. The chromatin network is prominent and many nuclei contain definite nucleoli. Some are small and pyknotic. The cytoplasm of these cells is somewhat pinker than that of the others. Occasionally large bone marrow giant cells are seen. In addition to these, occasional prominent multinuclear giant cells are seen with considerable pink-staining cytoplasm and three or four oval, prominent nuclei. The capillaries are engorged. Some of the tumor nodules appear to have an irregular fibrous tissue capsule about them but not completely encircling the nodules. The oxidase reaction is negative.

#### CASE 2.

*Clinical History:* H. S., age 71 years, was admitted to the medical service Aug. 11, 1929, complaining of pain in the lower spine, right shoulder, left chest, and inability to walk. Six months prior to admission, the patient was struck on the chest and developed a severe cough with bloody expectoration. On the following day he had severe pain in the lower spine and extremities and was forced to remain in bed for several weeks. The pain had persisted until the time of admission and during the last three days he had suffered knife-like localized constant pain in the shoulder and left chest. Polyuria and hesitancy were present for some weeks, though no dysuria. For several months he complained of weakness, dyspnea on slight exertion, orthopnea, cough and expectoration.

On examination he was pale and emaciated. His respirations were rapid. Over the right clavicle near the sternal border was a rather soft small protruding tender mass. His ribs were tender to percussion. A systolic murmur was heard

size, closely packed with round, somewhat vesicular nuclei and prominent nucleoli. Cytoplasm is slightly pink-staining and forms a syncytial mass with the surrounding cells.

Sections through the long bones show similar tumor masses in the bone marrow. In places the cells are polyhedral in shape. Mitotic figures are not prominent. In many of the cells the chromatin content is arranged about the periphery of the nucleus in cartwheel fashion. Apparently this appears to be a plasmocytoma.

*Kidneys:* The kidney has a very striking appearance (Figs. 4 and 5). There is an enormous increase in the interstitial connective tissue in the cortex, with marked destruction, atrophy and replacement of the tubular elements. The glomeruli show only an occasional atrophic change with hyalinization and thickening of Bowman's capsule. The glomeruli are otherwise intact. The capillaries of some of the tufts are congested. The tubules are strikingly atrophic and large areas are destroyed and replaced by what appears to be a densely cellular fibrous connective tissue. In addition there are small clumps of lymphocytes throughout the cortex. In areas the intact tubules are markedly dilated and filled with pink-staining material that has in places a lamellated structure. The central core is somewhat more pink-staining than the periphery. In the medulla many of the tubules show degenerative changes (Fig. 5). There is some increase in the interstitial tissue and many of the tubules are filled with pink-staining masses. Several of the tubules in the medulla contain masses of calcium in the lumina and in other places the lining epithelium contains calcium salts. The arterioles of the kidney show some thickening, and in areas, obliteration.

The picture in the kidney is that of very marked chronic nephrosis with extreme interstitial changes and shrinkage of the kidney. There is a concomitant arteriolosclerosis of mild degree. The glomeruli are only slightly affected. Calcification in some of the tubules of the medulla is present. The failure to find Bence-Jones protein in the urine is of no great significance as the patient was under observation for a week only, and it is well known that in the late stages of myeloma the Bence-Jones albumosuria may disappear.

*Kidneys:* The kidneys were small. Each weighed 100 gm. They were soft and yellowish in color. The capsules stripped easily, leaving a smooth surface. On section, the normal architecture was obscured and the surface had a homogeneous waxy appearance. The cortex was markedly narrowed, measuring 3 mm. in its widest portion. The pelvis of one kidney showed numerous petechiae. Ureters were patent.

*Intestines:* There were two small polyps in the lower part of the descending colon.

*Aorta:* The aorta showed very few raised white and yellow circular and oval atheromatous plaques and no ulceration, calcification or thrombi.

*Bones:* Several ribs were removed. The cortex was thinned out in areas. The marrow showed numerous grayish pink meaty nodules. Portions of the bodies of the lower lumbar vertebrae and of the left innominate bone showed extensive replacement of marrow by similar tumor tissue. The intervertebral discs were intact. The long bones were not removed. The brain and cord were not removed. The other organs showed no abnormalities.

#### MICROSCOPIC EXAMINATION

*Lungs:* There is edema and some emphysema in the lungs.

*Spleen:* The spleen is moderately congested. Follicles are not very prominent. A large amount of brownish pigment is present in the pulp tissue, primarily in the endothelial cells lining the sinuses. The arterioles show considerable thickening of the media and intima.

*Aorta:* The aorta shows typical atherosclerotic changes of the intima with calcification in the media.

*Prostate:* The prostate shows a variation in size of the glandular elements with increase in their number.

*Testes:* The testes show a marked increase in the interstitial connective tissue with atrophy of some of the seminiferous tubules and practically complete absence of spermatogenesis and replacement of interstitial cells of Leydig with connective tissue.

*Thyroid:* In the thyroid there is very marked calcification of the media of the arteries. Some atrophic changes in the lobules with an increase in the interstitial connective tissue are present.

*Bones:* In a section of rib, the bone marrow to a large extent is replaced by a very cellular mass. These cells are small, uniform in



and Epstein<sup>13</sup> both clinically and pathologically. In multiple myeloma there is true renal insufficiency characterized by a rise in the non-protein nitrogen of the blood, an inability to concentrate the urine with a corresponding low specific gravity. A large amount of protein is excreted in the urine (albumosuria and albuminuria), and the globulin-albumin ratio of the serum is reversed. Edema and hypertension are absent. Anatomically the nephrosis of myeloma proceeds to the end-stage of marked contraction of the kidney, and this extensive destruction of the tubules with fibrosis accounts for these differences in the clinical picture. Since concentration of the urine is said to occur in the tubules and since the tubules are destroyed in large numbers, the concentrating power is necessarily diminished and the specific gravity is low. With the destruction of large numbers of renal elements in the end stage of the nephrosis, the kidney function is strikingly impaired and even uremic manifestations may appear.

What relation does the Bence-Jones protein bear to the changes in the structure and function of the kidney? Is it possible for this protein substance to pass out of the blood through the normal kidney or is a previous injury to the kidney a necessary antecedent? And what relation does the Bence-Jones albumosuria bear to the severe degenerative disease of the tubules?

Anatomical kidney changes associated with Bence-Jones albumosuria are mentioned in most instances where the kidney pathology has been described in the literature. Of thirty instances with kidney lesions, about 65 per cent report positive Bence-Jones albumosuria. In about 25 per cent, Bence-Jones protein was not tested for, and in 10 per cent, no Bence-Jones protein was present. Of thirty-six instances where the kidney was described, thirty or 83 per cent report kidney lesions. Of these instances, twenty-seven described changes which in part are those of arteriolosclerosis of the kidney. In eight instances calcification of the tubules was reported. In six instances, a nephrosis was observed. In three instances, the arteriolosclerotic changes were associated with nephrosis, and in three instances the arteriolosclerotic changes were associated with nephrosis and calcification. Of eight instances with no gross kidney lesions, three were said to have Bence-Jones albumosuria, but in these cases there was no mention of the microscopic examination of the kidney.

## DISCUSSION

In both instances, a nephrosis complicated the multiple myeloma. The nephrosis was characterized clinically by the presence of large amounts of albumin and Bence-Jones albumose (in one instance) in the urine, with a low specific gravity, a low concentrating capacity, a rise in the non-protein nitrogen of blood and little edema. The severe secondary anemia is probably due to the bone marrow disease.

The changes in the kidneys in both instances reported were severe tubular destruction in the cortex and medulla with extensive replacement by connective tissue, interstitial lymphocytic infiltrations, casts in the intact dilated convoluted tubules of the cortex, and calcification of an occasional tubule in the medulla. The glomeruli showed slight hyalinization in some instances, but were for the most part anatomically intact. The picture was apparently that of a severe chronic nephrosis with evidence of secondary inflammation and fibrosis and contraction of the kidney. Since there is no certain microchemical method of detecting the presence of albumose in tissue sections, we were unable to determine whether the casts in the tubules contained albumose.

Frederich Müller<sup>10</sup> recommended the term nephrosis to indicate a primary degenerative disease of the tubules of the kidney. With the subsequent work of Aschoff<sup>11</sup> and Volhard<sup>12</sup> this disease entity was definitely separated from the so-called inflammatory diseases of the kidney. Etiologically true nephroses are produced experimentally in bichloride poisoning and in poisoning with chrome salts. Mild nephroses are observed in acute infectious diseases and occasionally in lues. The severest nephroses have been observed in patients in whom no etiological factor for the severe degenerative process could be determined.

In the idiopathic lipoid nephrosis there is no loss of the ability of the kidney to concentrate the urine in the tubules since these remain functionally active and the specific gravity is high. A large amount of protein is excreted in the urine, the globulin-albumin ratio of the serum is reversed and edema is prominent. Hypertension is absent and there is no rise in the non-protein nitrogen. The usual course is relatively acute and the kidney may recover anatomically. The chronic nephrosis of multiple myeloma differs from the idiopathic lipoid nephrosis first described by Müller, Volhard,

Jones protein can pass through the normal kidney in man. Though Bence-Jones protein may not ordinarily pass through the normal kidney, it is possible that the accumulation of large amounts of this substance in the blood of patients may produce functional injury to the kidney glomeruli and permit the excretion of the protein. On the other hand, since some arteriolosclerosis with glomerular injury is present in most individuals of the age group in which myeloma is found, it is possible that this degree of injury may be sufficient to allow of the excretion of Bence-Jones protein in the urine.

It is conceivable that the constant excretion of Bence-Jones albumose through a kidney previously injured, and its accumulation in the tubules, may be responsible for the subsequent nephrosis occurring in certain instances of multiple myeloma. It is possible that some other factor is responsible for both the nephrosis and the Bence-Jones albumosuria, and that some metabolic disturbance analogous to that occurring in true idiopathic nephrosis may be present in multiple myeloma.

Calcification observed in the kidney may be interpreted as part of the so-called metastatic deposits observed in diseases in which bone is extensively destroyed. Calcification of tissue in the kidney, the glandular mucosa of the stomach and the alveolar walls of the lungs was first noted by Bender<sup>18</sup> in an instance of myeloma, and subsequently by Tschistowitscht and Kolessnikoff,<sup>19</sup> by Froboese,<sup>20</sup> by Morse,<sup>21</sup> Thannhauser and Krauss<sup>6</sup> and by Charlton<sup>22</sup> and Kleine.<sup>8</sup> The calcification may be due to a general disturbance in calcium metabolism with hypercalcemia. However, the injury to the tubules probably predisposes to its deposition in the kidney. In one of the cases reported in our paper, there was a hypercalcemia.

#### SUMMARY AND CONCLUSIONS

Two instances of multiple myeloma with severe nephrosis and shrinkage of the kidney are reported. A review of the literature reveals several other similar instances. A study of the pathological changes of the kidneys in instances of multiple myeloma associated with Bence-Jones albumosuria is made. From this analysis it is concluded that the pathological changes found in the kidney of multiple myeloma consist of three distinct elements, no one of which is constantly present but all three of which may be present: (1) a

In a few instances, inflammatory lesions were noted in the kidney, but these were apparently complications such as pyelonephrosis or abscesses of the kidney. A true nephritis was described in only one instance, to the authors' knowledge. There can be no doubt that many of the instances described as chronic interstitial nephritis and arteriolosclerosis are cases of chronic nephrosis with contraction of the kidney. However, the presence of some degree of vascular disease of the kidney in most individuals over 55 years of age may have masked the nephrosis in such cases.

It is evident from this study that in almost every instance of multiple myeloma with Bence-Jones albumosuria there is present a disease of the kidneys which is obviously unrelated to the disease multiple myeloma *per se*, and unassociated with the nephrosis. The frequency of arteriolosclerosis of the kidney in the age group 50 to 70 years suggests that this lesion is a purely accidental finding.

Although it is possible that the Bence-Jones protein may pass through a normal kidney, proof of this fact cannot be obtained from the study of the pathology of the kidney in multiple myeloma. The evidence that Bence-Jones albumose can pass through a normal kidney is based on experiments in which Bence-Jones albumose was injected intravenously in rabbits and in dogs. It is well known that the injection of a foreign protein into an animal previously uninjected is followed by the appearance of that protein in the urine of the animal. It is argued by analogy that the Bence-Jones protein acts in the body as a foreign protein and is therefore eliminated in the normal kidney. Stokvis<sup>14</sup> induced kidney changes in dogs following the injection of Bence-Jones protein intravenously, and recovered the Bence-Jones albumose in the urine. Ellinger<sup>15</sup> was unable to confirm this finding. Von Decastello<sup>16</sup> found that a previous injury to the kidney was essential in permitting the appearance of Bence-Jones albumose in the urine in dogs injected with Bence-Jones albumose. Krauss<sup>17</sup> again succeeded in producing a nephrosis with Bence-Jones albumosuria in rabbits injected intravenously with Bence-Jones protein. However, he injected 1.25 gm. of Bence-Jones protein intravenously into himself, and was unable to recover Bence-Jones protein in the urine. When injected, however, into a patient with hemiplegia and evidence of arteriolosclerosis of the kidney, Bence-Jones protein was recovered in the urine. The experimental evidence does not permit the conclusion that Bence-

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## DESCRIPTION OF PLATES

### PLATE 64

- FIG. 1. Section through cortex of kidney of Case 1. Glomeruli intact, tubules dilated and filled with casts. Round cell infiltration of the interstitial tissue.
- FIG. 2. Section through medulla of the kidney of Case 1, showing marked dilatation of the tubules with casts and calcification of one tubule.
- FIG. 3. Section through medulla of kidney of Case 1. Extensive fibrosis with replacement of tubules. Round cell infiltration. Atrophy of remaining tubules.

nephrosis specifically associated in some way with the Bence-Jones albumosuria and the myeloma; (2) arteriolosclerosis of the kidney, an independent vascular disease of the kidneys, present in a milder or severer form in almost every instance of multiple myeloma occurring in the age group 50 to 70 years, and (3) calcium deposits in the kidney tubules dependent on a destruction of bone and the release of large quantities of calcium in the blood.

Clinically the nephrosis differs from the idiopathic type in the absence of edema and in the increase in the concentration of non-protein nitrogen in the serum, and an inability to concentrate urine with a consequently low specific gravity to the urine. Anatomically the nephrosis is severe and the kidneys contracted. The kidneys are small and pale with a smooth surface and markedly narrowed cortex. Microscopically there is extensive destruction of the tubules of the cortex and medulla with replacement by dense cellular fibrous tissue. Though there is some hyalinization of a few glomeruli, this lesion is apparently associated with a concomitant arteriolosclerosis, the primary lesion being the destruction of the tubules.

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PLATE 65

FIG. 4. Section through cortex of kidney of Case 2. Glomerulus shows slight atrophic change but is relatively intact. Marked dilatation of the convoluted tubules with flattening of the epithelium. The tubules are filled with casts.

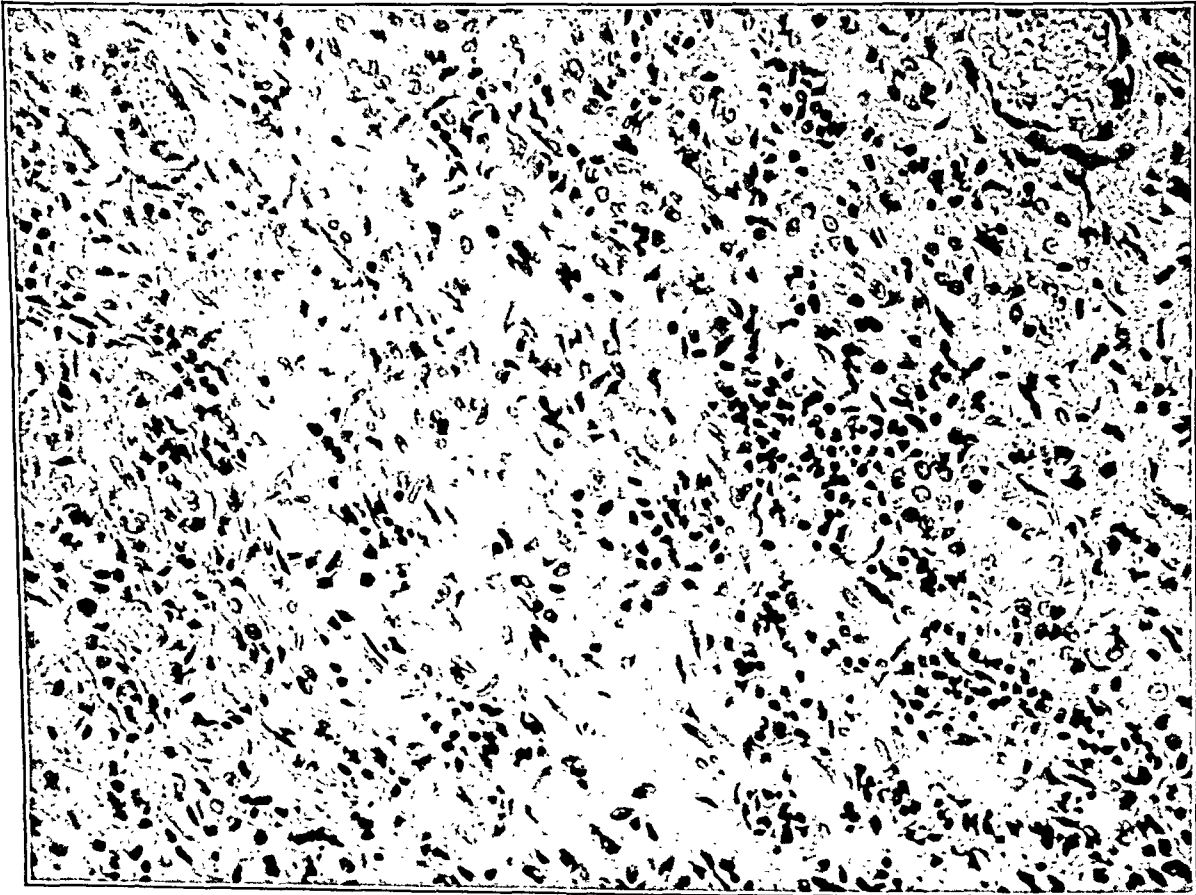
FIG. 5. Section through medulla of kidney of Case 2, showing extensive fibrosis with destruction of tubules and round cell infiltration. Intact tubules are dilated and filled with casts.



1



2



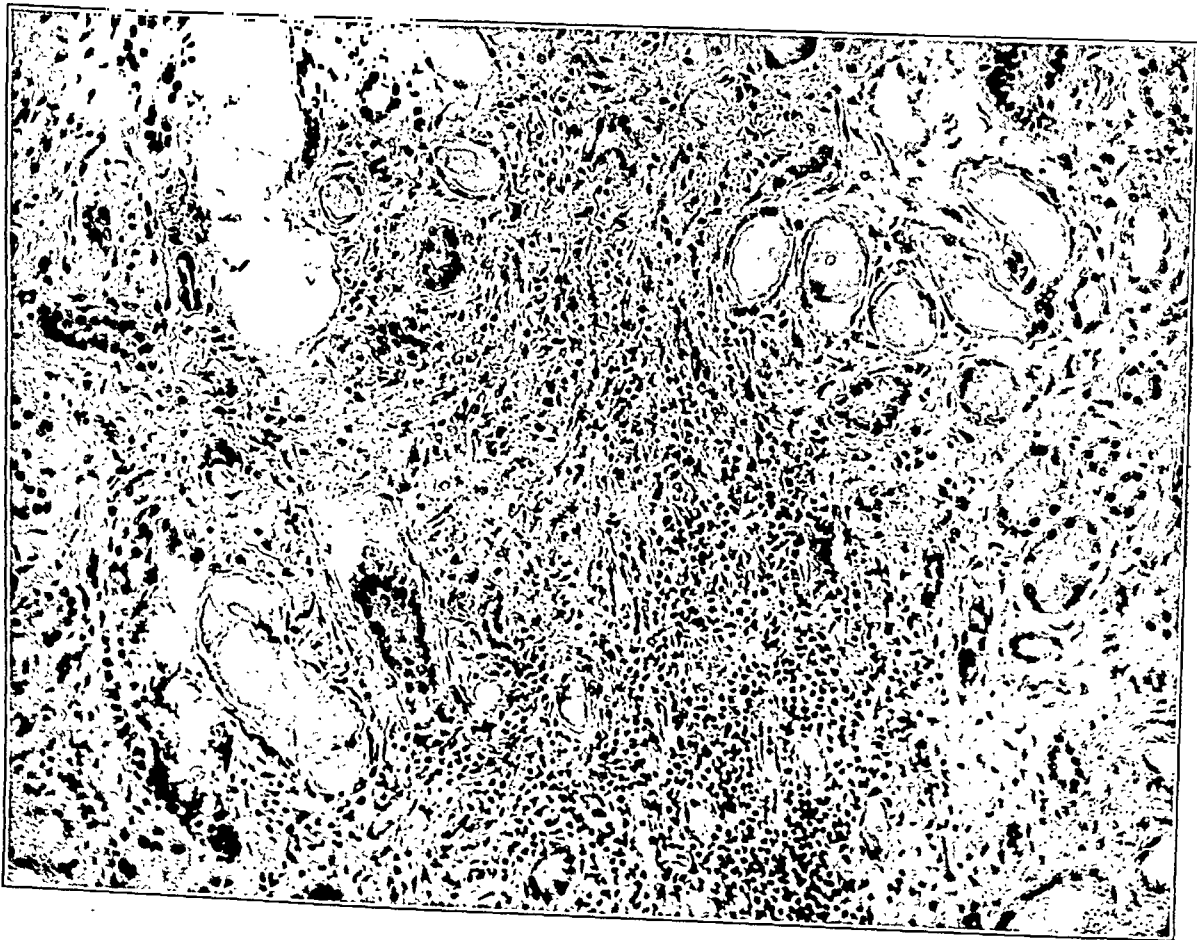
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5

Atanasoff<sup>3</sup> says: "Grains, especially rye, in some seasons and in some fields are infected so heavily that nearly every head may have one or more sclerotia, as was reported from Wisconsin in 1917. Cases where 20 to 50 per cent of the heads were infected with ergot have been reported from various parts of the United States and Europe. The amount of ergot for 1917 in Connecticut ranged from 1 to 5 per cent. Decrease of the yield [of rye] by as high as 20 per cent has been reported from Russia." (As a result of ergot infection.)

The importance of the present-day ergot infection of rye can be judged by the defensive measures used by the grain farmers to remove the fungus. Many methods, such as sifting, screening, fanning and sedimentation are employed, all depending on the difference in size and weight of the grain compared to the fungus. It is considered that with careful cleaning of the seed the ergot can be diminished to from 0.06 to 0.17 per cent. It is of course not expected that such a diminution could occur where the ergot amounted to 5 or more per cent of the grain. From personal experience with twenty samples of rye flour obtained from grocers and the New York Produce Exchange in 1925, I found anywhere from five to twenty-five flecks of ergot on a microscopic slide. Considering the amount of ergot-infected rye bread consumed here and abroad it is surprising that cases of endemic ergotism are so rarely reported. It may well be that such conditions as thrombo-angiitis or Raynaud's and other vasomotor and trophic diseases have been overlooked as possibly being sequelae to ergot poisoning. This may be because of the mildness of the original intoxication and the insidious onset of the vascular changes.

In studying the nationalities and races affected with thrombo-angiitis obliterans, we find the disease most prevalent in people from those countries where rye bread is the staple article of diet, generally in the Northern Slavic countries — Russia, Poland, Ukraine, East Prussia and Lithuania. Most of the cases reported by Buerger<sup>4</sup> and Jablons<sup>5</sup> in New York City were immigrants from those countries. Buerger, who is responsible for much of the literature and experimental work on this disease and who has given it the name thrombo-angiitis obliterans, believes that the disease is peculiar to the Jewish race. In his large series of cases in New York City, patients were almost exclusively Jewish. Jablons reported

## THE PATHOLOGICAL SIMILARITY OF THROMBO-ANGIITIS OBLITERANS AND ENDEMIC ERGOTISM \*

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Thrombo-angiitis obliterans and ergotism (gangrenous form) have many points in common. This is particularly true of their vasomotor, trophic and sensory symptoms and signs. Even though the histological descriptions in ergotism are meager, they show a resemblance in the important pathological characteristics; namely thrombosis with obliteration of the vessel lumina.

The cause of thrombo-angiitis obliterans is unknown, and if we are to consider ergot poisoning as the cause we must first know some facts about the probability.

It is well known that epidemics of ergotism were due to the eating of bread made of ergot-infected rye. These epidemics have been traced back as far as Galen, circa A.D. 200. For a complete history of epidemic ergotism up to 1888, the works of Krysinisky<sup>1</sup> and Allbutt and Dixon,<sup>2</sup> from whose works I have borrowed freely in this article, are recommended. There have been very few epidemics of any importance in the past 100 years because of the greater care, cultivation and separation of the ergot sclerotia from the rye grains. In spite of our knowledge and care there have been isolated epidemics as late as 1908. This is not at all surprising when we realize the quantity of grain that is known to be infected with the ergot fungus in our present day.

Since the ergot fungus attacks most of the grains and grasses, it is not strange that we have reports of its presence in most portions of the globe. As rye is the most susceptible host, it is natural to find this fungus most prolific in those regions where rye occurs in the greatest abundance. It has been reported to occur in every continent including Australia and New Zealand. In North America it has been found in Vermont, Connecticut, New York, Michigan, Tennessee, Indiana, Wisconsin, Minnesota, Iowa, Kansas, Wyoming, Montana and other states, besides various places in Canada.

\* Received for publication January 27, 1930.

pertinent that both thrombo-angiitis obliterans and gangrene ergotism have a preference for the male sex.

*Age:* Thrombo-angiitis obliterans occurs most frequently in young and middle-aged men. In epidemic ergotism the gangrenous form, according to Krysinsky, occurred most frequently in young and middle-aged men.

*Social Class:* Thrombo-angiitis obliterans occurs principally amongst the laboring classes. This was also found true of epidemic ergotism. The explanation given is that rye bread, which was the cheapest form of food, was the principal article of their diet.

*Diet:* As the chief article of diet in the North Slavic countries is rye bread, it is most natural that the patients coming from those countries should admit eating rye bread. It is also natural for the poor laboring classes, who make up the largest proportion of thrombo-angiitis obliterans cases, to be big consumers of this cheapest form of food. Those patients whom I questioned, admitted eating large quantities. One-half to three-quarters of a pound of rye bread during a meal was not considered unusual. Rye bread as we know was also the universal diet of the patients afflicted with epidemic ergotism. Those who ate liberally of meat, milk and cheese suffered less severely, or not at all. The addition of meat and milk to the diet of people who consume large quantities of maize, serves to prevent the occurrence of pellagra. This additional diet may serve in a similar way to neutralize the toxins of ergot. Those people who eat much rye bread might be advised with profit to add meat and milk to their diets.

It is well known that large quantities of rye bread are consumed by the Germans, yet there are relatively few cases of thrombo-angiitis obliterans reported by them. It may be that they are more careful in selecting and cleaning their grain, or perhaps their more varied diet helps to prevent the disease. Another element to consider is the preparing of the bread. In the Slavic countries a sour bread is made of rye flour leavened with old rye dough instead of yeast. The dough is allowed to ripen each time for several days. It takes much longer than with yeast. A small quantity of dough is always saved for the next baking. It is possible that a rye flour rich in ergot might develop larger quantities of the so-called putrefactive substances such as histamin and tyramin if allowed to stand

90 per cent Jews in a series of 200 cases in New York City. He mentions Idelson reporting 60 per cent Jews in a series of 226 cases from Poland, Lithuania and East Prussia. He quotes various other authors as reporting cases in Norwegian fishermen in Norway, Scandinavians in Minnesota, and Japanese and Chinese in their natural habitat. It would seem from this that thrombo-angiitis obliterans is more widespread than originally thought by Buerger. It is probable that the large percentage of cases reported in any particular race depends to a great extent on the character of the author's experience. In considering the susceptibility of the Jews to this disease, it is significant that this disease is very rare in the descendants of native Jews of the United States, England and Germany, whose dietetic habits are different from the Slavic Jews. It seems more rational to regard the susceptibility as one depending on habits and susceptibility peculiar to the individual, rather than to any racial idiosyncrasy.

*Sex:* Of 500 cases reported by Buerger three cases occurred in females. In Jablon's 200 cases, 1 per cent occurred in females. Idelson<sup>6</sup> reported 14 women in a series of 358 cases. The greater susceptibility of males to thrombo-angiitis obliterans is very evident from these figures. One can find a parallel here in the susceptibility to the gangrenous form of ergotism which affected the males to the greatest extent. In sixty-eight epidemics of ergotism, according to Krysinisky, the gangrenous form affected, with one exception, the males exclusively. The convulsive form affected the women, children and the aged. Renaudin<sup>7</sup> stated that the gangrenous form of ergotism does not affect females. Tessier<sup>8</sup> observed that spurred rye acts with less force on females. What is true of epidemic is also true of medicinal ergotism. In spite of the tremendous quantity of ergot consumed in this country<sup>9</sup> — 170,000 to 264,000 pounds imported yearly — one rarely ever hears of a case of gangrene in women. It is interesting to note that even in fowls we encounter an increased susceptibility in males. Gittinger and Munch<sup>10</sup> in an assay of ergot by the cock's comb method, found that the hens had no value, their combs showing slight blanching or no effect whatever; while, of the control, 90 per cent of the cocks gave satisfactory results.

It is not within the realms of this paper to consider why certain diseases are limited to one or the other sex. For our purpose it is

In comparing gangrene ergotism and thrombo-angiitis obliterans we note that we have in both, symptoms and signs of a vasomotor and trophic condition. We have in both conditions pallor, rubor and cyanosis; pains, paresthesias and contractions in the legs; imperceptible pulse, trophic changes, ulceration and gangrene.

It is interesting to note that the patients, outside their local symptoms, appear to be in splendid health in both these vascular diseases.

### COURSE

The course of thrombo-angiitis obliterans is very chronic, lasting many years. It is not known how soon the intoxication occurs before the symptoms. In ergotism, symptoms of gangrene have been noticed to occur several months after the original intoxication. In horses, Buffum<sup>11</sup> noticed gangrene to occur two years after the initial ergot intoxication. If such late manifestations occur in humans it is not strange that the original intoxication is lost sight of. Such may also be a possibility in thrombo-angiitis obliterans. It is possible that susceptible individuals, by daily feeding with rye bread slightly infected with ergot, may not show acute manifestations of ergotism but later develop the chronic manifestations of thrombo-angiitis obliterans.

### PATHOLOGY

Only some of the salient features of the pathology of thrombo-angiitis obliterans will be described here. For details on this subject, Buerger, Mahorner,<sup>12</sup> and Ramirez<sup>13</sup> are recommended.

The lesions of thrombo-angiitis obliterans are generally found in the extremities and occasionally in the viscera. They are in part due to neurotrophic disturbances, but principally to a vascular occlusion which leads to infarction. The typical lesions are found in the blood vessels, which become thickened and thrombosed with obliteration of their lumina. The veins are often the seat of acute thrombophlebitis. This acute venous inflammation and thrombosis has led Buerger to apply the name thrombo-angiitis obliterans to this disease. It was his opinion that the disease began with an acute inflammation and thrombosis of the vessel. Earlier authors, Friedländer,<sup>14</sup> and some of the more recent authors, Ramirez and Mahorner, believe that thrombosis is secondary to an intimal proliferation.

for a long time. The Germans are not as partial as their Slavic neighbors to this sour rye bread.

To explain the occurrence of thrombo-angiitis obliterans in the Chinese and Japanese, from the standpoint of ergotism, one would have to know more about the diet of these people. It may be that their rice had been infected by ergot. In the more Northern districts where rice does not grow, rye bread forms a big part of their diet.

### SYMPTOMS

For the symptoms of gangrenous ergotism we are forced to rely on the reports of the epidemic form as the reports of the endemic form are practically limited to the medicinal intoxications. After the acute symptoms, such as headaches, vomiting, cramps, diarrhoea, etc., agonizing pains appear generally in the legs. These pains, which are said to penetrate the limbs like fire, both precede and accompany the gangrene. The limb becomes livid, which in some cases is preceded by an erysipelatous blush. The cyanosis passes into darkness and blackness, and finally gangrene, which may be either dry or moist, generally the former.

The artery is felt to tighten from day to day and the pulse becomes very small until finally it is imperceptible. Amongst the sensory symptoms mentioned are formication, anesthesia, coldness, heat and pain. Tonic contractions of the legs are also mentioned. There may be pallor, rubor, or cyanosis. Gangrene may follow months after the initial intoxication.

The chief symptoms of thrombo-angiitis obliterans are burning or cramp-like pains in the calf of the leg. There is generally present paresthesia, anesthesia, formication, coldness or heat. There may be pallor, cyanosis or rubor. When the affected leg is elevated it immediately becomes bloodless, but soon changes from pallor to the original redness or cyanosis when placed in the pendant position. There are trophic changes to be noticed in the skin and nails of the fingers or toes affected, to which may be added ulceration and gangrene. Occlusion of the artery is essential to the diagnosis of thrombo-angiitis obliterans. The symptoms occur in some cases long before the complete thrombotic occlusion. This is particularly true of the vasomotor and sensory symptoms and suggests the possibility of recurrent spastic contraction of the diseased vessels.



quently found to be denser on account of the increased connective tissue. Many of the nerves are found in a state of degeneration. This is attributed to the ischemia and not correlated with any cerebrospinal changes. Whether the cerebrospinal tract is affected by this disease is not known as no autopsy report of its investigation exists.

### PATHOLOGY OF ERGOTISM

We have unfortunately very meager information of the pathology of ergotism. We are familiar with the text-book's description of a contraction and thickening of the vessel wall and a thrombosis of the lumen followed by gangrene of the extremity. Dr. Mitchell Bruce<sup>15</sup> says that the posterior columns of the cord show sclerosis. In the constricted and thrombosed arterioles a glutinous matter is found and the vessels either primarily or secondarily undergo hyaline degeneration, especially of the tunica intima.

As in thrombo-angiitis obliterans, the visceral vessels may also be affected. Perhaps the most detailed pathological report on ergotism in humans is the one by Vinogradoff<sup>16</sup> on the epidemic of acute convulsant ergotism that occurred at Viatka in 1889. Only the viscera were found affected, principally the liver, kidneys and spleen. Nearly all the large vessels of these organs contained thrombi. Some were red, others contained fibrin with scattered erythrocytes. In smaller branches of the portal vein organized thrombi were found. Some vessel walls were thickened, their lumina narrowed to such an extent as not to permit more than two or three erythrocytes to pass. Marked intimal proliferation was observed in some vessels. Many of the vessels had undergone fibrosis or hyaline degeneration. In spite of the more acute lesions described in these cases of ergotism, it is possible to correlate some of the vascular changes with those occurring in thrombo-angiitis obliterans.

As I could not obtain pathological sections of chronic ergotism for my studies, I attempted to produce this condition in animals. In my early experiments I employed the usual laboratory animals: mice, guinea pigs and rabbits. I found these rodents very resistant to ergot and had to change to roosters, which have long been known to develop gangrene of the comb after the ingestion or inoculation of toxic doses of ergot.

Some of the pathological data of three ergotized roosters will be

The latter believes that even the acute thrombophlebitis of this disease is a superimposed condition, other vascular changes having preceded it.

The theory of the gradual narrowing of the lumen by the encroaching thickening intima is more consonant with the gradual impairment of the circulation noticed in this disease. An acute inflammation accompanied by a sudden thrombosis in an artery would produce very acute local manifestations with gangrene at the outset of the disease before collateral circulation could be established.

It is a question whether it is wise to draw definite conclusions of the pathogenesis in the arteries from the findings in the acutely inflamed veins. Acute arterial inflammation is relatively rare, whereas acute phlebitis is common. This is not so surprising when we realize that the veins, particularly of the extremities, receive tribute directly from the superficial tissues, frequently the seat of infection.

Bacteria have frequently been found in thrombo-angiitis obliterans but none has so far been proved to be specific to this disease. In the absence of this evidence and the fact that the patients rarely have any of the constitutional symptoms of bacterial diseases, one should seriously consider as a cause an intoxicant capable of inducing the pathological changes of this disease.

In following the pathological progress in the vessels one might suggest that an initial intoxication, by injury or only severe excitation, results first in a contraction of the walls and in intimal hyperplasia (Fig. 1). The second phase is the clot which becomes attached to the intimal process. Both this intimal process and the red clot undergo organization and often fuse so thoroughly as to make one fibrous mass. One is then unable to identify with any certainty what is intima, thrombus, or both (Fig. 2).

As organization takes place, various areas of the vessel would become impoverished unless some compensatory circulation were established. Small vessels are formed in the fibrotic mass and media to offset the impaired circulation. These new vessels are not found in the adventitia which perhaps has an independent circulation. These new vessels are one of the characteristics of the later phases of the disease.

Arteries are frequently found with their accompanying veins and nerves adherent to the adjacent structures. These nerves are fre-

engorged with old blood. There is evidence of marked stasis. On either side of the vascular stalk there is condensation of the mucoid tissue with a disappearance of the nuclear elements: this condensed tissue stains quite blue with the hematoxylin and eosin stain.

The erectile tissue in the periphery of the mucoid tissue and just under the epithelium is greatly in evidence. There are more blood spaces and there is more engorgement. The papillae of the epithelial covering are flattened by this engorgement. There is some edema in the epithelium and the epidermal layers are increased.

Section D. Area of dry gangrene; tip of posterior portion of the comb, distal to the line of demarcation. The stalk area is composed of condensed mucoid tissue, the nuclei of which are in a good state of preservation. The vessels in the stalk are markedly engorged with blood. The erectile tissue around the stalk is very much in evidence, the blood spaces being markedly engorged with blood. In some of these spaces are to be found black amorphous masses, which are evidently degenerated blood pigment. In some portions there is early vacuolization in the subepidermal layers of the squamous epithelium. It would seem that this picture suggests attrition of tissue, and early stage of dry gangrene.

A similar picture is present microscopically in the section proximal to this, in which the gross picture is not yet that of necrosis. This description coincides with the gross pathology, which suggests a pregangrenous condition.

Cock No. 25: White leghorn cock about 1 year old, weight 500 gm. This case is of a more chronic duration, about seven months, the animal having been fed 180 gm. of ergot intermittently for four months. It developed vasomotor and trophic disturbances of the comb two months after the initial feeding.

*Autopsy:* The only gross lesion to be found is in the comb which is about twice its natural thickness. It is purple at its tip and pale at the base. Its entire surface is covered with small dry grayish scales and numerous fine capillary hemorrhages.

*Pathological Histology:* Section through the middle of the posterior portion of the comb. There is no change in the vascular stalk. Some of the arteries show vacuolization in the adventitia. Some of these vacuoles are large and lined with endothelioid cells; some of these are found throughout the vascular stalk. They suggest chronic lymphedema. One of these vessels shows marked intimal

described here. The first one, although not of chronic ergotism, is described to illustrate how acute a process may be as a result of a change in the vascular tone, and also on account of its possible bearing on the acute lesions of thrombo-angiitis obliterans where no bacteria have been found. Complete protocols will be published elsewhere.

### EXPERIMENTS

Cock No. 24: Hybrid, red and white leghorn cock about 7 months old, weight 1250 gm., fed 28 gm. powdered Russian ergot in four days. Died of exhaustion on the ninth day after the initial feeding.

*Autopsy:* The animal appears emaciated, weight 880 gm.; the comb is very dark purple, the posterior tip is dry, hard and black (dry gangrene). There is a line of demarcation about 1 cm. from the tip. The border of the wattles, the breast and the rump is discolored with a dark hemorrhagic rash. On section of the skin the purpuric rash is seen to extend through to the pectoral muscles and into them. There is very little subcutaneous or omental fat. Heart normal; lungs normal, except for a small area of reddish consolidation at the left base; liver has a grayish infarct about 5 cm. in circumference.

*Gross Pathology:* Gangrene of the comb; purpura of comb, wattles, skin of pectoral and rump regions; consolidation of the base of the left lung; infarct of the liver.

*Microscopic Examination:* Lesions of acute ergotism found in sections of the base of the posterior portion of the cock's comb.

Section A. The larger arteries are somewhat contracted; the intima is puckered up. There are some spaces (fat globules) in the intima and subintimal regions. The intima stains in a somewhat hyaloid manner and has some vacuoles in its midst (Fig. 3).

Section B. About the middle of the posterior portion of the same comb. The central stalk shows very marked hyperemia. The larger vessels are engorged; many small vessels are visible which are not present in the preceding section. The arteries are dilated, their coats stretched thin. There is no evidence of endarteritis nor of any lesion of the media.

Section C. Near the tip of the posterior portion of the comb, proximal to the line of demarcation (Fig. 4). The central vascular stalk shows condensation of the fibrous fatty tissue. The vessels are

distinct thrombus which is apparently firmly attached to one side of the intimal ring and apparently in a stage of organization. The media and adventitia are comparatively uninvolved. This lesion has all the apparent marks of the condition called thrombo-angiitis (Fig. 6).

*Microscopic Examination:* Several other arteries of the vascular stalk are found to have the same intimal lesions, but in none of these are there attached organizing thrombi (Fig. 7.). These latter correspond to endarteritic lesions. The mucoid tissue is unchanged except toward the epithelium where it is condensed. Here the erectile tissue shows some diminution in the size of the blood spaces. The squamous epithelium is somewhat condensed and the epidermis is in greater evidence.

It might be argued that the chronic endarterial changes in this animal resulted from the injury caused by acute ergotism. In the preceding animal, however, marked intimal proliferation occurred as a result of chronic intoxication. It may be that chronic changes occur as a result of either acute or chronic ergot intoxication, very much as in bacterial intoxications.

If we were to consider some of the essential pathological changes found in both human and experimental ergotism, such as thickening of the vessel, particularly the intima, and the thrombus formation with organization, we might argue the identity of ergotism with thrombo-angiitis obliterans. Complete occlusion with canalization of the thrombus which was absent in ergotism might occur in cases of longer standing. Several years are perhaps necessary to develop this chronic process. An acute condition superimposed on a chronic one may be due in ergotism and thrombo-angiitis obliterans to a re-intoxication with the causative toxins.

## TOXICOLOGY

Ergot, *Claviceps Purpurea*, is a fungus that attacks most of the grains and many of the grasses, but its most common host is rye. The fungus quite early in the life of the rye plant incorporates itself into the rye heads and becomes a part of certain of the grains. The vicarious grains appear somewhat larger than the normal grains on the same head, besides being considerably darker than, and easily distinguished from, the rye grains. These abnormal grains are

thickening with great vacuolization of the pathological endothelium, which is irregularly heaped up to many layers in all but one small sector.

*Microscopic Examination:* The mucoid layer is markedly thickened: the fibrillae are greatly separated and their nuclei are few in number. This is all suggestive of chronic edema. A great many cells of the midlayers of the dermis show marked vacuolization, the nuclei standing out in the periphery of these spaces. This suggests chronic lymphedema. The epidermis appears more granular than usual, and is separated from the dermis by a distinct layer containing spaces (Fig. 5).

This animal developed vasomotor and trophic changes in the comb without having developed acute symptoms. Some of the vessels showed chronic endarterial changes with narrowing of the lumina. The marked stasis, edema, etc., could not be accounted for by the findings in these vessels which were not actually occluded. We can only deduce that these vessels which were in a state of spasm as a result of ergot feeding continued in this spastic state for three months after this drug was discontinued.

Cock No. 23: Plymouth Rock about 15 months old. This case is that of a rooster which was fed about 250 gm. of ergot over a period of one year. He was fed large doses in the seventh month and developed very acute symptoms such as weakness, dyspnoea, diarrhoea, etc. There was a necrotic process on the middle tip of the comb, the latter being purplish in color. An ulceration appeared at the base of the neck in front, which was found to communicate with the gullet from where much of the food was expelled. This fistulous tract was closed up with sutures and ergot was fed to him again until the fistula reopened about five months later.

As we are primarily interested in the affected vessels and the tissues they supply, only the comb will be considered in the autopsy of this animal.

*Autopsy:* The comb is slightly swollen, dry and rough. It is contracted to one side and has a nodule firm to the touch, 3 mm. in diameter, at its junction with the beak.

*Pathological Histology:* The central stalk shows one of the arteries supplying the surface of the comb to be the seat of marked change. There is an extensive heaping up of the intima which is markedly vacuolized, giving the appearance of fatty deposits. There is a

sels by perfusing them with ergot, such effects have been obtained by perfusion with histamin.<sup>17</sup>

The motor effects of ergot are of two types, a chronic contraction of the uterus and in the skeletal muscles (epidemic convulsive ergotism), and a tonic contraction such as occurs in the vascular muscles leading to gangrene. These two different motor effects may be due to the individual substances present in ergot, such as ergotamin, tyramin or histamin, which account for the variation in the types of epidemics, the gangrenous or the convulsive form of ergotism predominating depending on the predominant toxin.

Albutt and Dixon ascribe the possible differences in the strength and action of the various ergots to the ground on which they grow. They compare ergot in this respect with *Papaver somniferum* in that the same opium grown in Turkey has a richer yield in morphin than when grown in England. It is quite possible that certain yields of ergot also contain more or less histamin and tyramin depending on the country where they were grown. In the foregoing experiments, I obtained my best results with Russian ergot. The relative content of ergotamin, histamin and tyramin in the ergot of this country, Russia, Poland and Spain may prove of interest and importance.

The seasons and the "freshness" of the ergot also deserve consideration. The Abbé Tessier,<sup>18</sup> commenting on the conditions likely to promote an epidemic of ergotism, said "that the district was damp, that the vegetable products were ill thriven and stunted, that the inhabitants were in bad health, being reduced by want and malaria." Salerne<sup>19</sup> proved with experiments in animals that fresh ergot, which was most toxic, lost its toxicity in a few months.

Guggisberg<sup>20</sup> maintains that both histamin and tyramin occur only in old ergot and are not found in perceptible quantities in fresh ergot.

As has been mentioned, a sour rye bread is made in which the dough is allowed to leaven for several days, without yeast. Whether the ergot present might elaborate in this dough increasing quantities of histamin and tyramin or cholin bodies, and so become more toxic is a matter of importance and should be determined.

We have considered how ergotamin, histamin and tyramin, by a combination of arterial constriction and capillary dilatation and stasis, lead to acute gangrene. The angiospasm which induces

known as sclerotia and are a stage of the life cycle of the ergot fungus.

The growth and abundance of the ergot sclerotia is dependent to a large extent on moisture. The epidemics were observed by many authors to occur after a damp spring. It is probable also that the dampness may also influence the quantity and type of toxin elaborated by the fungus, for it has been noticed that in certain regions and at certain periods the gangrenous form of ergotism predominated, while in others the convulsive form was in greatest evidence.

Ergot is not the simple substance it was originally thought to be. Even today the pharmacologists are not quite agreed upon the active principals of this drug. Briefly it may be stated that in addition to its chief active substances, ergotamin and ergotoxin, there are found relatively small quantities of so-called putrefactive substances, histamin, tyramin and cholin bodies. The action of ergotamin and ergotoxin can best be described as inhibitors of the motor sympathetic fibers. This is best proved in their counteracting the glycemic and pressor action of adrenalin. Contrary to the accepted belief, ergotamin does not constrict the blood vessels. This phenomenon is most likely due to the other substances in ergot, histamin and tyramin. Tyramin is a very powerful arterial constrictor, whereas histamin, although it has slight arterial constricting qualities, is chiefly a capillary dilator. The combination of these three substances, ergotamin, histamin, and tyramin, undoubtedly favors the production of gangrene. Tyramin by constricting the arteries, and ergotamin and histamin by dilating the capillaries cause a marked stasis which eventually leads to gangrene.

Both tyramin and histamin produce uterine contractions. The latter in dilution of one to a hundred million produces vigorous contractions of the frog's uterus. Histamin when injected in a cock intramuscularly will cause a very prompt darkening of the comb. This phenomenon is much more vigorous and quicker than when whole ergot is employed. It appears from this that the present cock's comb method of assaying ergot in this country is faulty, since it does not distinguish between its principal alkaloids and one of its so-called impurities — histamin.

The action of whole ergot takes place perhaps through the central nervous system, and, to some extent, local stimulation. Although Albutt and Dixon did not succeed in diminishing the caliber of ves-



## SUMMARY

We have shown:

1. That both thrombo-angiitis obliterans and ergotism (gangrenous form) occur most frequently in people of the same sex, age and social status.
2. That the symptoms and physical signs may be the same in both conditions.
3. That the pathological findings in both may be the same in the earlier stages.
4. That the main article of diet in both conditions is rye bread.
5. That ergot is a common infection of all grains, particularly rye, in every continent of the globe.

NOTE: I wish to acknowledge my obligation to Dr. Irving Simon for the microscopic descriptions, and to Dr. A. A. Eisenberg for the facilities of the pathological department of the Sydenham Hospital.

Grateful acknowledgement is also made to Mr. and Mrs. Herbert M. Rothschild for their splendid coöperation.

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trophic changes also accounts for such phenomena as intermittent claudication, local syncope and asphyxia, cramping pains in the legs, etc., as occur in arteriosclerosis, Raynaud's disease, thrombo-angiitis obliterans and ergotism.

As has been suggested, the acute reactions that recur in the vessels of thrombo-angiitis obliterans may be due to an allergic response to ergot. If this suggestion is to be given consideration, then histamin, one of ergot's principal ingredients, should first be thought of because it is already looked upon with suspicion as the biochemical culprit of anaphylaxis.

### CONCLUSIONS

There is much evidence in favor of an identity of thrombo-angiitis obliterans and ergotism. There are also some dissimilarities, but they can be ascribed to a difference in chronicity.

Ergotism should be produced in animals, preferably mammals, with a view to obtaining a chronic process of at least several years duration, for comparison with thrombo-angiitis obliterans, Raynaud's disease, erythromelalgia, sclerodactyl, etc. The results obtained in roosters, although suggestive, are too far removed biologically for definite comparison.

Careful investigation should be made of the cerebrospinal tract in all these vasomotor and trophic diseases. The cord has been neglected in the study of thrombo-angiitis obliterans.

A study should be made of the difference in the histamin and tyramin content in addition to the ergotamin in the ergot from various countries. Such studies should also be made of rye breads leavened in the ordinary way with yeast, and with old dough. Studies should be made with these ergot substances alone, and in various combinations on the effect on the vessels. Studies should be made with these substances from the allergic standpoint on the vessels, particularly histamin, which is a suspected substance in anaphylaxis.

It is hoped that these studies will lead to the prevention of some vasomotor and trophic diseases whose origin is not known.

## DESCRIPTION OF PLATES

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### PLATE 66

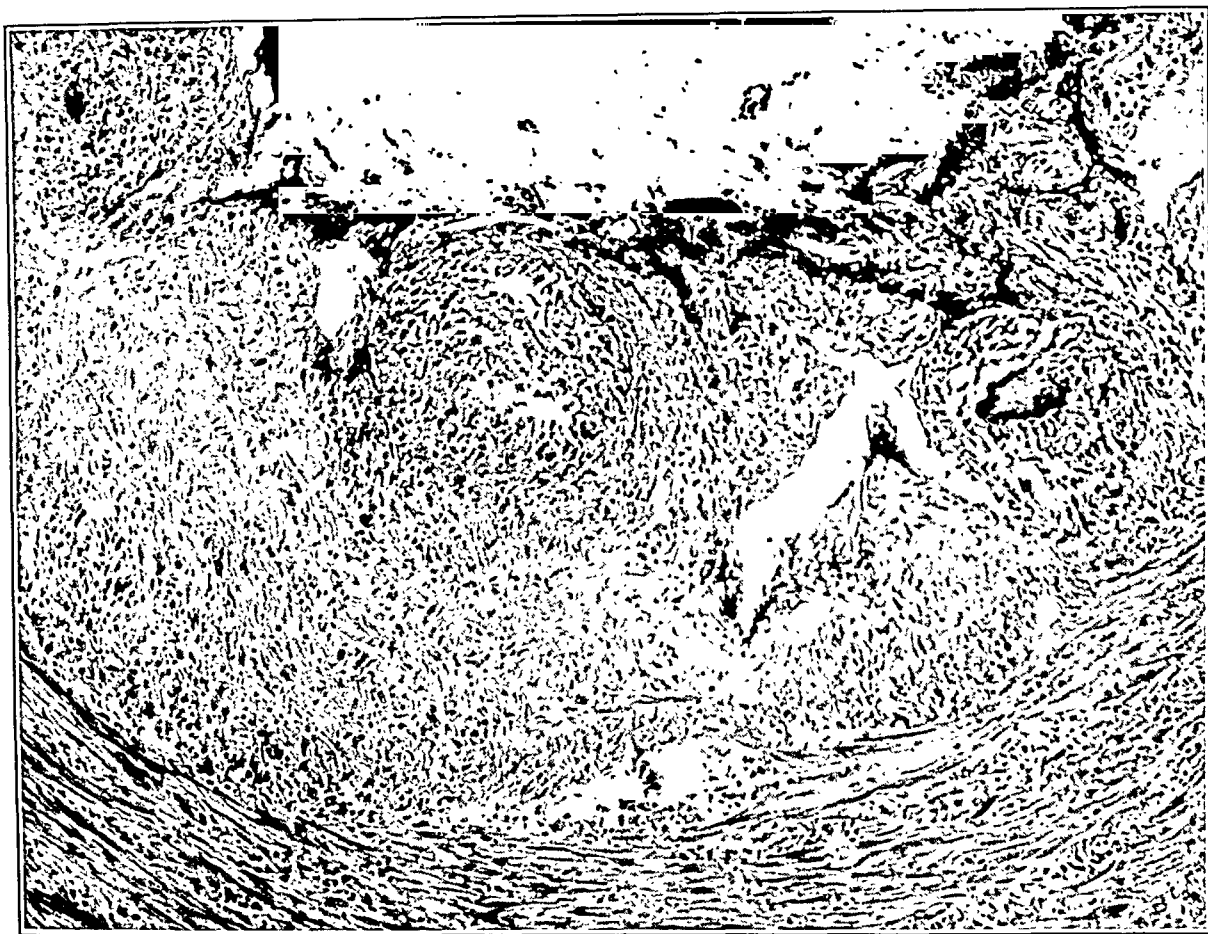
FIG. 1. Early stage of thrombo-angiitis obliterans, showing marked hyperplasia of the intima, to which is attached a recent thrombus. Note the invasion of the thrombus by the intimal cells. This will result in a fusion of the thrombus with the hyperplastic intima.

FIG. 2. Thrombo-angiitis obliterans in three vessels illustrating three later stages. To the right a vessel is shown to which a thrombus has very likely been attached to the hyperplastic intima, with which it became fused and organized so that there is now no means of identifying the two processes. To the left is a vessel in which the hyperplastic intima is the important feature. The vessel in the middle illustrates the end process of thrombo-angiitis obliterans.

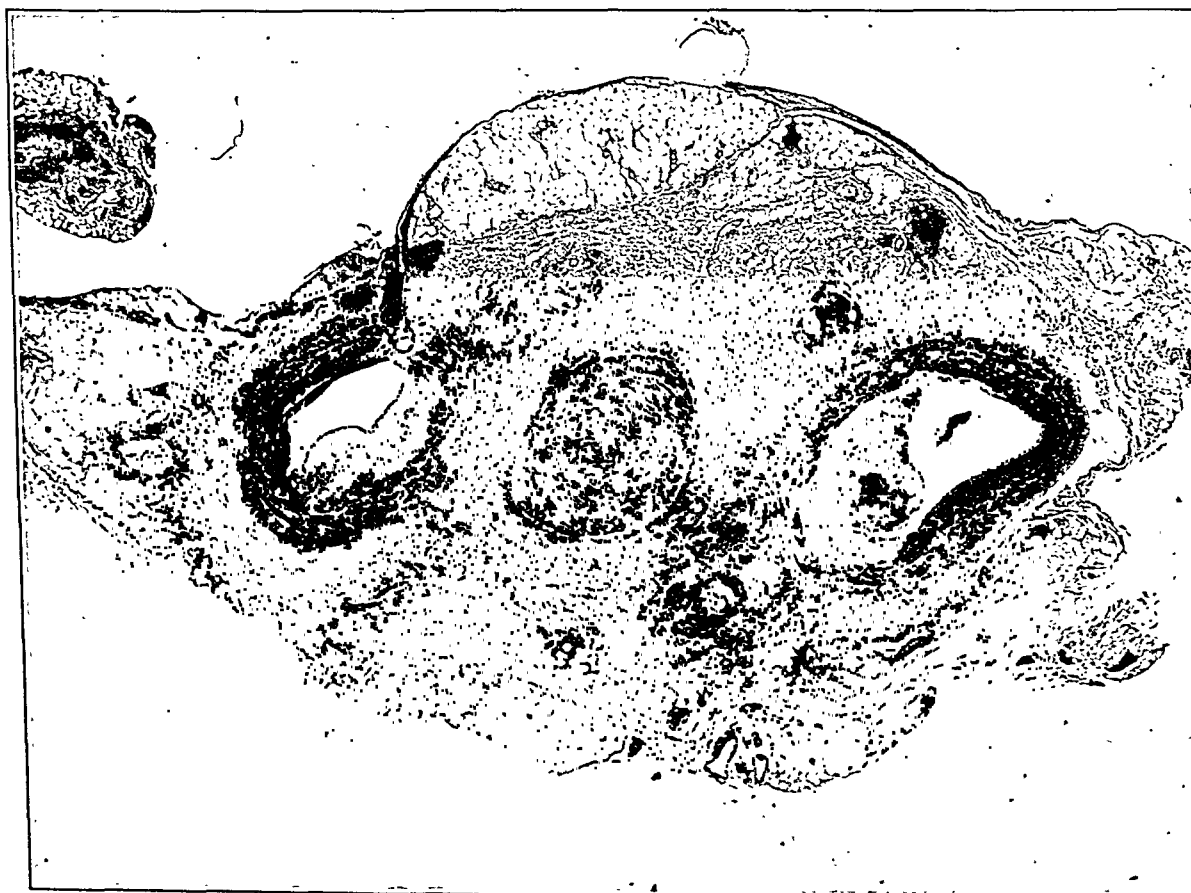
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PLATE 67

- FIG. 3. Acute ergotism in an artery at the base of the posterior portion of the cock's comb showing marked thickening of the vessel and contraction of the lumen. The muscularis appears edematous and stains poorly.
- FIG. 4. Acute ergotism of the cock's comb near the posterior tip showing marked dilatation of the vessels and stagnation of all the tissues with old blood.



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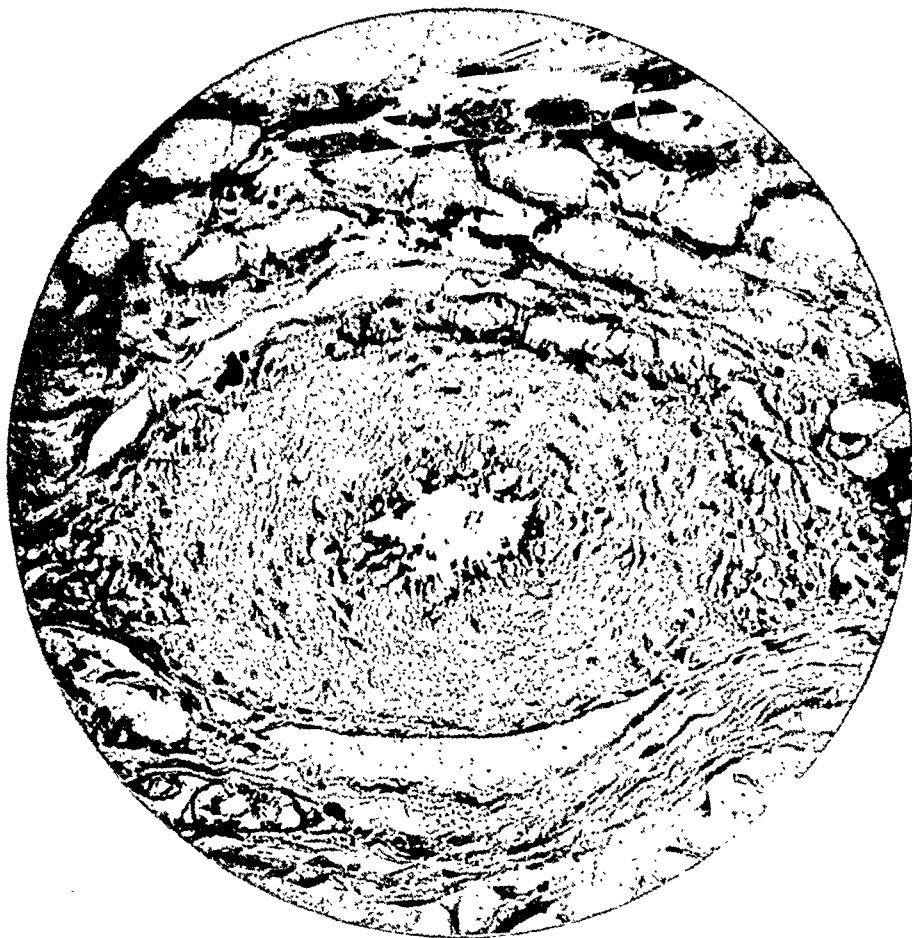


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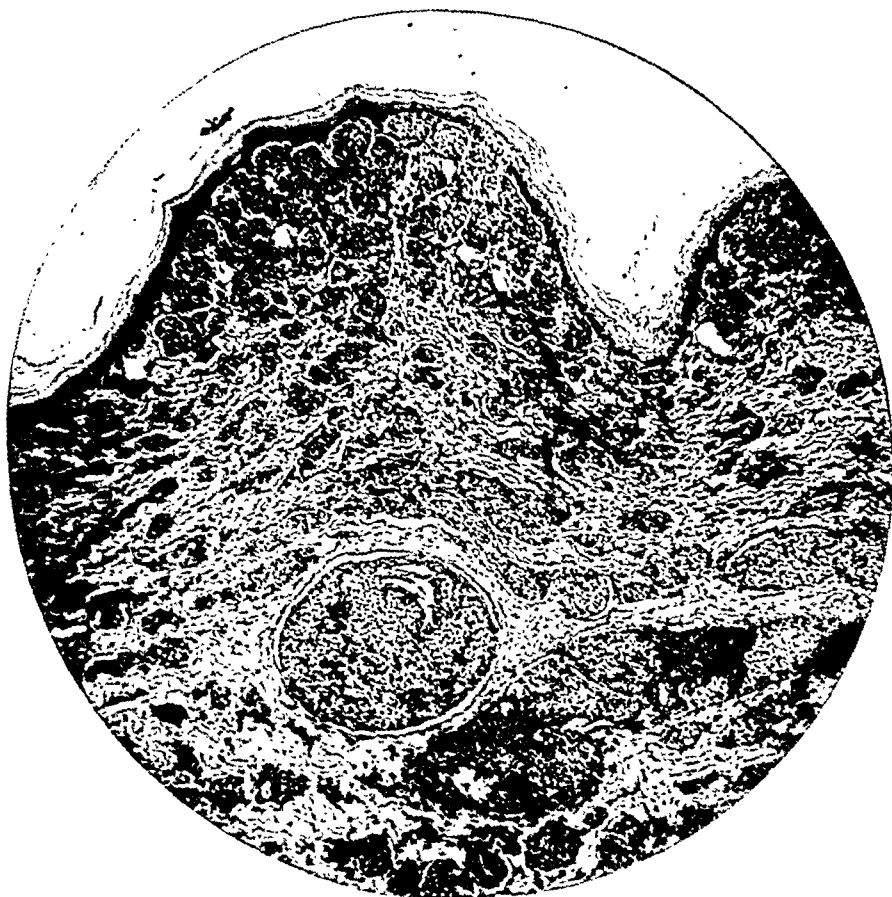
PLATE 68

FIG. 5. Chronic ergotism of the cock's comb showing edema of the epithelial layer and desquamation of the thickened epidermis.

FIG. 6. Chronic ergotism in a vessel of the cock's comb showing marked intimal hyperplasia with an adherent thrombus which is fused and organized.



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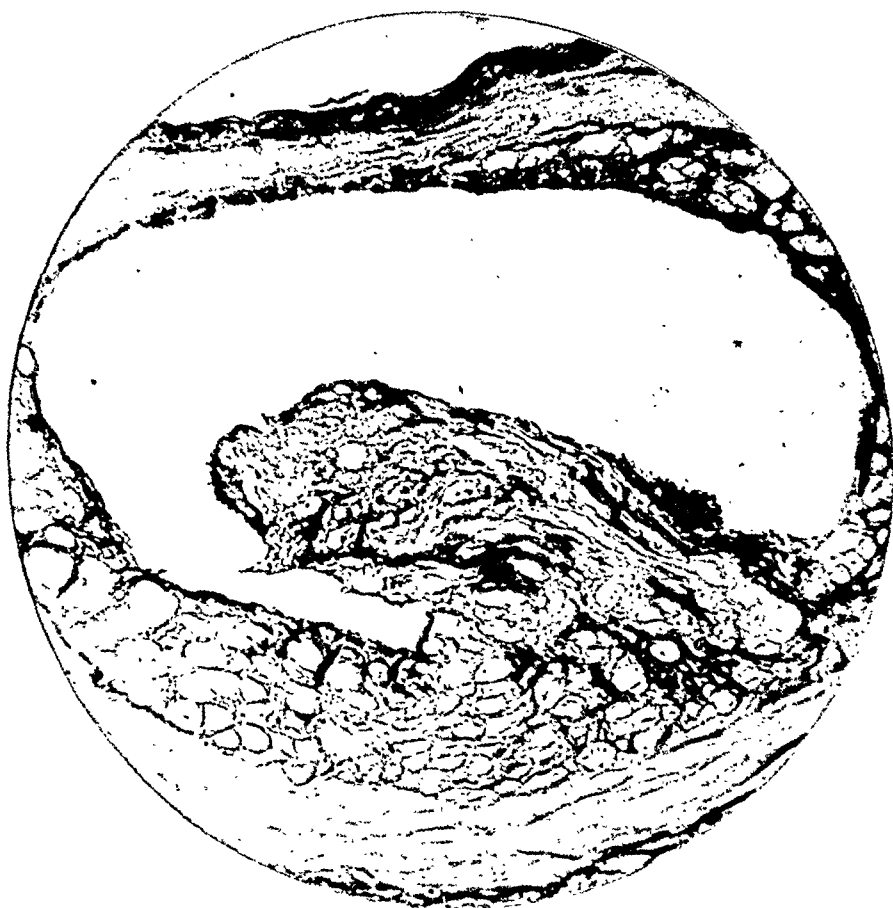
PLATE 69

FIG. 7. Chronic ergotism, cock's comb. Marked proliferation of the intima in an artery of the median stalk. Hematoxylin and eosin stain.  $\times 50$ .

FIG. 8. Transverse section of a normal cock's comb. (1) Median vascular stalk. (2) Muroid area. (3) Vascular erectile tissue. (4) Epithelial covering. Hematoxylin and eosin stain.  $\times 10$ .



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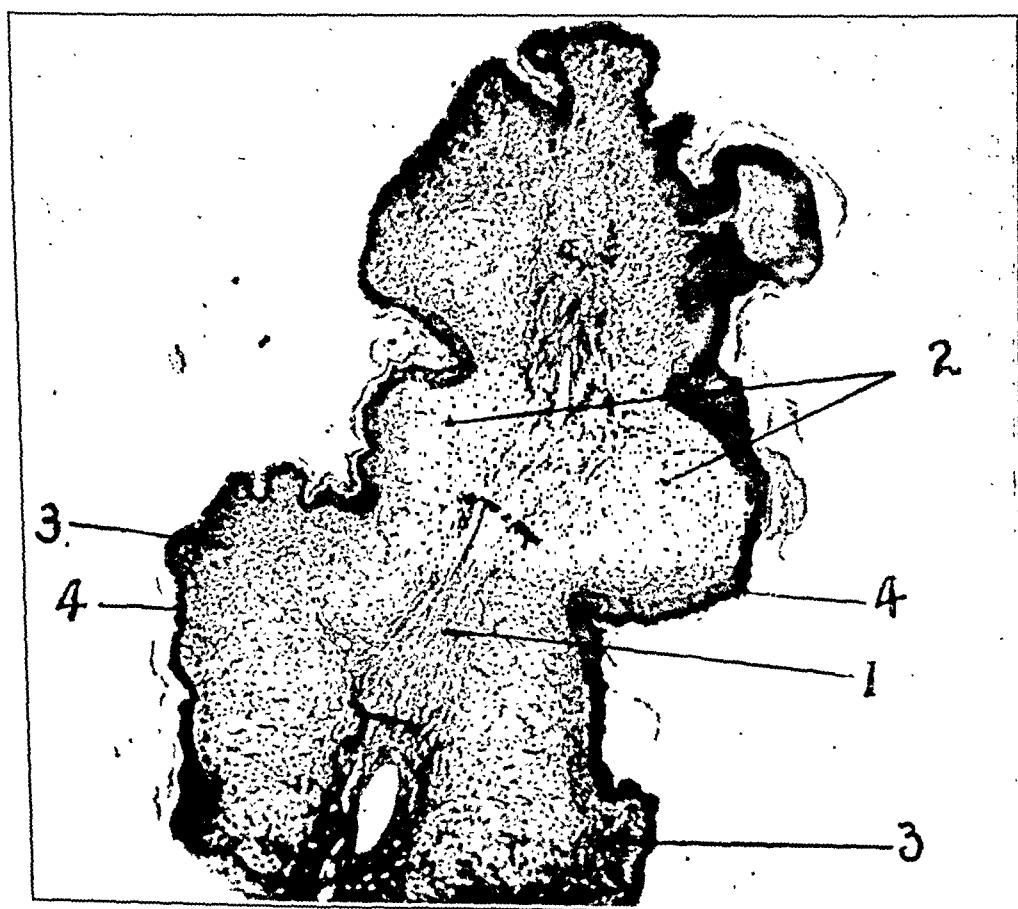


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Presystolic and systolic thrills were felt over the apex and at the left sternal border at the level of the third and fourth costal cartilages. The heart sounds were regular and the rate 60 beats per minute. The first apical and second pulmonic sounds were accentuated. Along the left sternal border, high pitched diastolic and systolic murmurs were audible. The blood pressure systolic-diastolic was 90/60 mm. Hg.

The monthly dispensary visits of the patient until her first entrance to the hospital revealed no changes in her condition. In 1923, at 17 years of age, she was admitted to the hospital for the first time complaining of shortness of breath on the slightest exertion. Physical examination disclosed cyanosis and malnourishment, with clubbing of the fingers and toes. Additional signs in the heart were systolic and rumbling diastolic murmurs at the apex. During her five weeks' hospitalization the cyanosis decreased but never entirely disappeared. The clinical diagnoses at this time were: (1) congenital heart disease, patency of interventricular septum, and (2) rheumatic heart disease, with mitral stenosis and insufficiency.

Following her discharge, the patient continued to make frequent visits to the dispensary. Her only new complaints were occasional precordial pain and swelling of the ankles.

In May 1929, at 22 years of age, and one month prior to her second entrance to the hospital, she felt a localized sharp epigastric pain of ten minutes duration. One week later an upper respiratory infection developed and was accompanied by considerable coughing without sputum. She entered the hospital, complaining of a cold and chills. The teeth contained many fillings and several cavities. The tonsils were greatly enlarged and a thick yellow mucoid material covered the posterior pharyngeal wall. The signs in the heart were unchanged except for a blowing systolic murmur heard over the pulmonic area. The infection abated and she was discharged in three days.

Two months later for the third time she reported to the hospital because of chills and joint pains which had begun ten days before. The pain was most marked in the left knee-joint and there was also swelling of the left ankle. The patient, as previously noted, was malnourished and cyanotic and further appeared acutely ill. The signs in the heart, found on previous admissions, were unchanged

A CONGENITAL ANOMALY OF THE HEART  
(TRUNCUS ARTERIOSUS COMMUNIS WITH SUBACUTE  
ENDOCARDITIS) \*

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INTRODUCTION

Truncus arteriosus communis is one of the rare types of cardiac malformation. Abbott,<sup>1</sup> in a recent article, reports twenty-three cases of this anomaly from the literature and other sources. One case has since been reported from this laboratory by Zimmerman.<sup>2</sup> The embryological factors responsible for this abnormality are discussed in detail by Abbott and Shanly.<sup>3</sup>

An individual with truncus arteriosus communis seldom lives beyond the period of childhood and rarely develops an intercurrent infection of the heart. Lesions similar to those found in this case are therefore unusual. It is well known, however, that intercurrent cardiac infections are common in many other types of congenital heart diseases. The case here reported is of special interest because of the valve injuries, the age of the patient and a ten-year period of observation in the medical dispensary and ward services.

REPORT OF CASE

*Clinical History:* The patient, a female, was under observation at the New Haven Hospital and Dispensary for a period of ten years prior to her death at 22 years of age. In 1919, the patient, then 12 years of age, reported to the dispensary complaining of nervousness and palpitation. She was born a "blue baby," was always cyanotic and physically could not exert herself beyond such moderate exercise as walking. Physical examination revealed cyanosis and malnourishment. The cyanosis was general over the face, hands and feet. The finger tips were markedly clubbed. The left border of cardiac dullness extended out to the anterior axillary line and the apex impulse was felt 11 cm. from the midsternal line.

\* Received for publication December 19, 1929.

extremity is tense, shining, and pitting edema is present throughout. Upon opening the abdominal cavity about 60 cc. of clear amber-colored fluid is found. The liver extends well below the costal margin. When the chest plate is removed, it is found that the transverse diameter of the heart is greatly increased, measuring 13 cm., while that of the thorax measures 19 cm. The pericardial surface is smooth and the pericardial sac contains about 30 cc. of a clear amber-colored fluid. The apex of the heart is blunt and the distance from the apex to the base is relatively short, giving the heart as a whole a spheroidal shape (Fig. 1). One large vessel only arises from the base, at the usual site of the origin of the pulmonic and aortic arteries (Fig. 2). The heart and lungs are, therefore, removed *en masse* together with the upper three-fourths of the thoracic aorta.

*Heart:* The heart is large, firm and estimated to weigh over 600 gm. A normal amount of subepicardial fat is present along the course of the coronary arteries. The right auricular appendage is large and passes well around the front of the common truncus arteriosus. No pulmonary artery or ductus arteriosus are found. The base of the common truncus measures 9 cm. in circumference. At its junction with the transverse portion of the vessel it gives rise to the right innominate, left common carotid and left subclavian arteries, in the usual manner. Beyond this point the circumference of the vessel decreases in size, measuring 6 cm. From here the vessel follows the normal course of the thoracic aorta and, aside from the vessels to be described, gives rise to the arteries usually derived from this vessel. Distal (1.5 cm.) to the origin of the left subclavian artery, the common truncus gives rise to a small artery measuring 5 mm. in diameter (Fig. 1, No. 1). This vessel passes to the right of and posterior to the trachea, entering the upper lobe of the right lung. On the same side, 3 cm. beyond this point, another vessel of the same diameter arises (Fig. 1, No. 2). This also passes to the right, just below and posterior to the bifurcation of the trachea, to enter the middle lobe of the right lung, dividing into two branches as it passes to this lobe. Beyond this vessel 1 cm., another artery is given off from the left side of the common truncus and runs to the hilum of the left lung (Fig. 1, No. 3). Just beyond the point of entrance into the lung it divides into three branches, one passing into the upper, and two into the lower lobe. At the

except for an additional long, blowing diastolic murmur over the pulmonic area. The temperature was  $104.5^{\circ}$  F, the pulse 135 per minute and the respirations 25 per minute. The blood pressure was 80/50 mm. Hg. On July 7th, the fourth day after admission, the spleen was palpable on deep inspiration. Blood cultures taken on three different occasions showed heavy growths of *Streptococcus viridans*. On July 26, three weeks from the date of hospital entrance, the patient complained of pain and numbness in the left leg and was unable to move her toes. Pulsation of the arteries of this extremity could not be felt. Cyanosis of the left leg rapidly increased. On July 28th, she became delirious and stuporous; the temperature rose to  $106^{\circ}$  F, the respirations became rapid, and the patient died on the afternoon of the same day.

The laboratory findings were: red blood cells 6,500,000 (the red blood count was found constantly high on all three hospital admissions), hemoglobin 120 (S), white blood cells 26,000 with 84 per cent polymorphonuclears and 13 per cent lymphocytes. The urine contained a heavy trace of albumin with 9 to 12 white blood cells per high power field. Electrocardiogram showed normal sinus mechanism with an average rate of 100 per minute (Chart 1). The chief ventricular deflections were downward in Lead 1 and upward in Lead 3. The auricular waves in Leads 2 and 3 were quite large and pointed. The T waves were upright in Leads 1 and 2 and diphasic in Lead 3. Diagnosis: normal mechanism. Left axis deviation.

The final clinical diagnoses were: (1) congenital heart disease, patency of the interventricular septum, rheumatic heart disease with mitral stenosis and insufficiency; (2) endocarditis — subacute bacterial; (3) embolism — left femoral artery.

#### POSTMORTEM EXAMINATION

The autopsy was performed three hours after death; only the findings bearing on the subject are included.

The body is poorly developed, emaciated and weighs 106 pounds. The mucous membranes, the nail beds and ears are deeply cyanotic. There is marked clubbing of the terminal phalanges of the fingers and toes. The entire left gluteal region and left lower extremity have a gray-purple, mottled appearance. The skin covering this



are prominent and deep. The right coronary artery arises in the left posterior sinus of Valsalva and the left coronary artery in the anterior sinus of Valsalva. Both coronary openings lie unusually high in the sinuses. On the anterior semilunar cusp is a large vegetation measuring 2.5 by 1.5 by 1.5 cm. (Fig. 2). It is of a greenish red color and has a granular fibrinous appearance. Its base is solid and firmly adherent to the ventricular surface of the cusp. The free portion of the mass is soft and friable. It lies for the most part in the right ventricle and almost completely fills the interventricular defect. Three or four small openings are present near the free edge of the posterior cusp. The intimal lining of the common truncus is smooth throughout. The mitral leaflets are delicate and velamentous and the chordae tendineae insert well beyond the free edges.

From the above description it is seen that the venous blood in the right heart is mixed with the arterial blood of the left, the admixture from both ventricles passing directly into the common truncus arteriosus. This common truncus with its mixed arterial and venous blood distributes a small portion to the lung by way of the pulmonary branches described above, and to the systemic circulation in the usual manner. The return flow from the systemic and pulmonary circulations passes into the left and right auricles respectively in the normal manner, and from the auricles through the usual orifices into the communicating ventricles.

*Spleen:* The spleen is large and firm. It weighs 500 gm. The capsule at the lower pole is roughened by a fibrinous deposit. The underlying splenic tissue has a dark purplish color as contrasted with the deep red normal-appearing surrounding parenchyma. A cross-section through the center of this lesion and the hilum reveals a cone-shaped area of opaque darkly colored tissue with the apex of the cone toward the hilum, and the base, measuring 6 cm. across, beneath the capsule. An inelastic friable clot is found in a branch of the splenic artery supplying this region.

The lower portion of the left common iliac artery with its branches, the left external and internal iliac arteries, are non-compressible on palpation. Cross-section through these vessels shows their lumina to be entirely occluded by a dark reddish brown inelastic mass.

The following anatomical diagnoses were made at the completion of the autopsy.

posterior medial aspect of the lower lobe of the right lung is found still another vessel passing from the organ at this point (Fig. 1, No. 4). This measures about 5 mm. in diameter; it is thin-walled and is probably a vein passing out of the substance of the lung. Its point of termination is destroyed on removal of the organ. The truncus, at the level of the third thoracic vertebra, just below the pulmonary vessels described, measures 4.5 cm. in circumference.

The right auricle is approximately two-thirds as large as the left. The foramen ovale is closed. In general, the wall of the right auricle is thicker than that of the left by 1 mm. The superior and inferior vena cavae, the coronary and pulmonary veins, open into the auricle in the usual manner. The right ventricular wall measures 13 mm. in thickness and the left ventricular at its corresponding level measures 11 mm. The average thickness of the interventricular septum is 14 mm. In the upper portion of the septum is a defect measuring 2.5 by 1.5 by 1 cm. (Fig. 2). The semilunar cusps of the common truncus lie immediately over this opening. The anterior and posterior boundaries of this defect are formed respectively by the anterior and posterior ventricular walls; the lower boundary is formed by the ventricular septum. There appears to be no remains of the pars membranacea. The common truncus arteriosus arises, therefore, directly over the defect and communicates with both ventricles. The cavity of the right ventricle is nearly twice as large as that of the left. The mural endocardium of all four heart cavities is smooth and glistening. The papillary muscles and the columnae carnae stand out prominently from the ventricular walls. On the tricuspid valves are two healed vegetations which project 1 to 2 mm. above the surface of the valve leaflets on their auricular surfaces. The larger mass measures 4 by 5 mm. and lies on the posterior leaflet; the smaller one measures 2 by 3 mm. in diameter and is situated upon the median leaflet. Most of the chordae tendineae of the posterior leaflet arise directly from the interventricular septum in the upper portion. Those of the median leaflet arise from the lower portion of the septum, while the chordae tendineae of the posterior leaflets arise from the papillary muscles of the right ventricular wall, beneath and posterior to the valve defect. The semilunar cusps of the truncus are three in number and large, formed by a right and left posterior and anterior cusp. The sinuses of Valsalva

DESCRIPTION OF PLATES

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PLATE 70

*Primary:* Healed tricuspid endocarditis, acute vegetative endocarditis (valve of the common truncus), infarcts of the spleen, embolus in the left common iliac artery.

*Subsidiary:* Truncus arteriosus communis persistans, ventricular defect, cardiac hypertrophy, Meckel's diverticulum, accessory spleen.

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PLATE 71

FIG. 1. A 1862. Posterior view of heart and lungs showing common truncus and origin of pulmonary arteries to lobes of lungs.

FIG. 2. A 1862. Longitudinal section of heart showing common truncus arteriosus and interventricular septal defect.

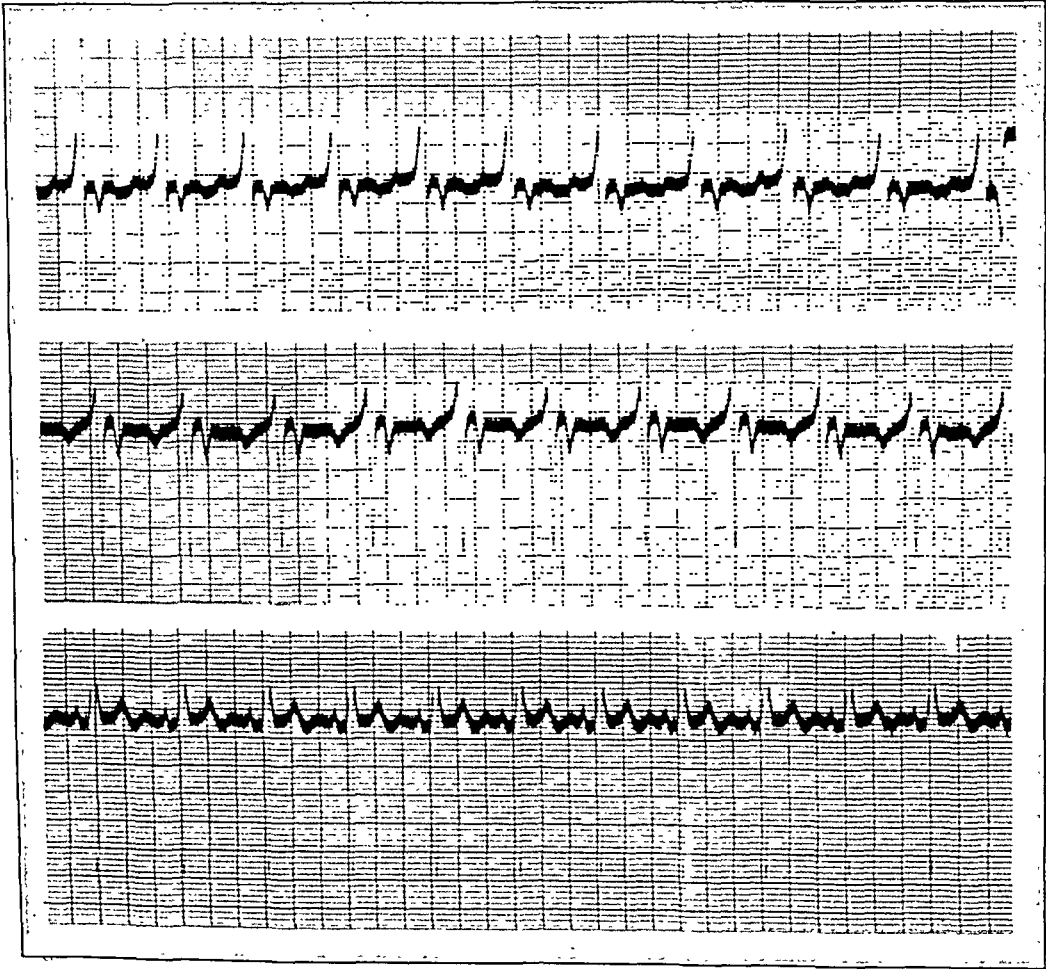
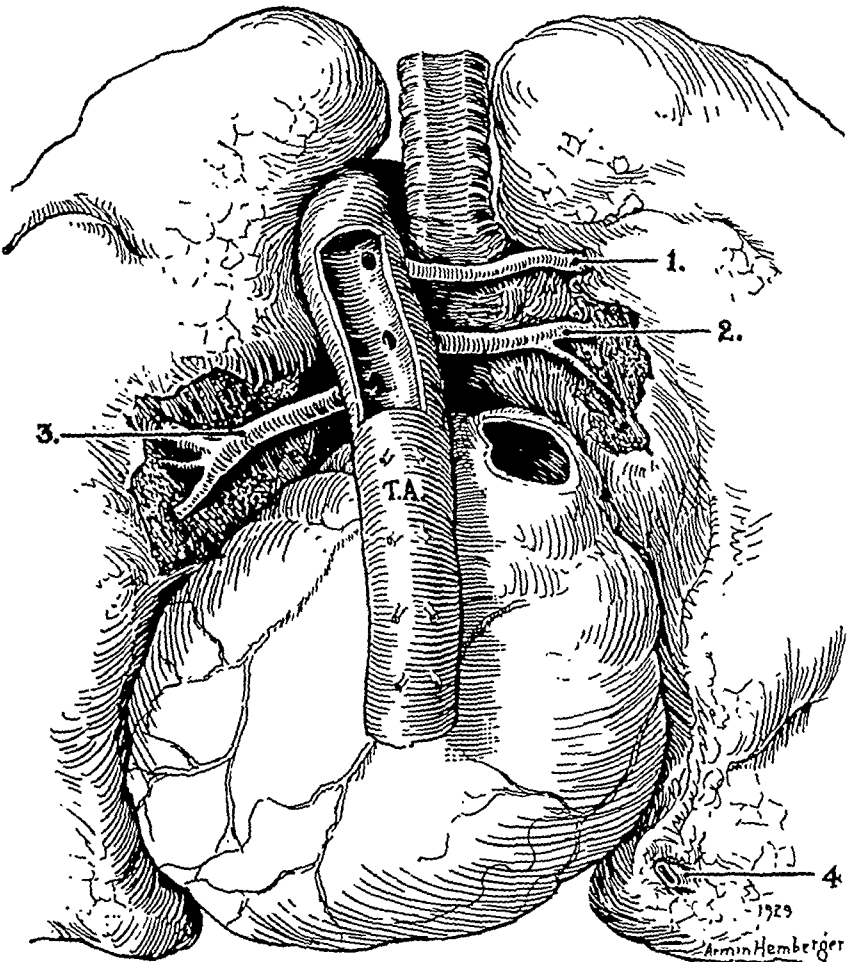
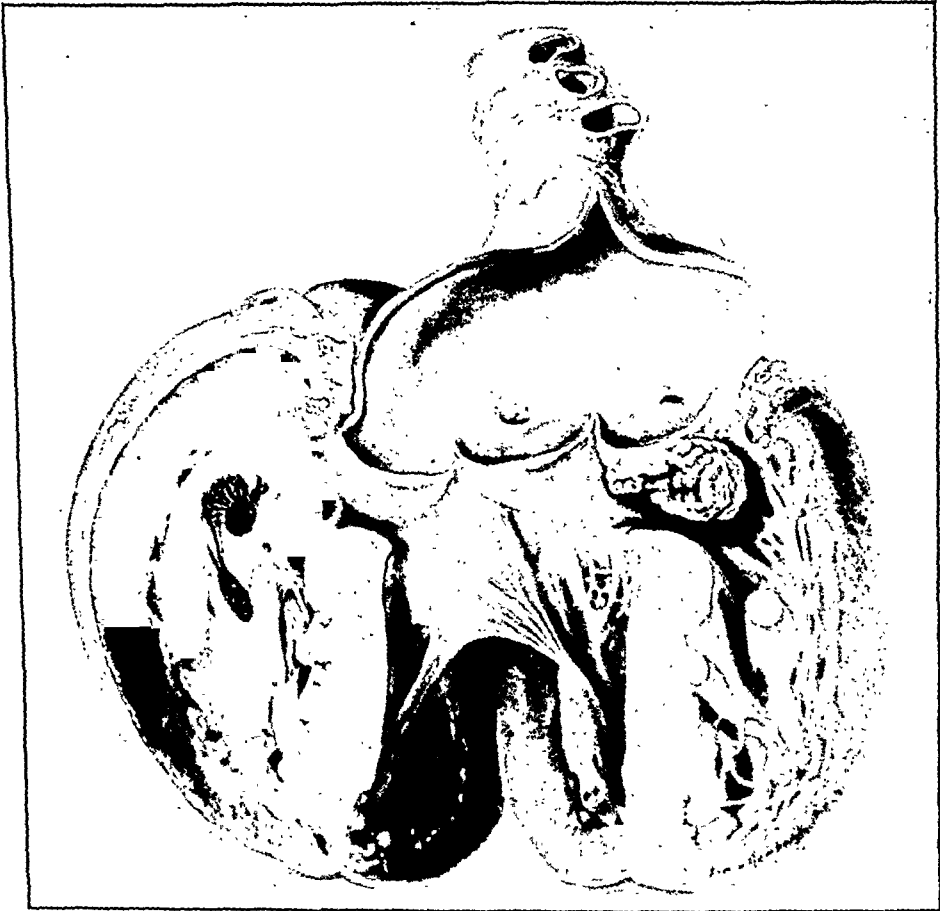


Chart 1





1



2



## REVIEW OF LITERATURE

Tarozzi<sup>4</sup> isolated and described an organism which he at first mistook for an actinomycete causing a chronic osteomyelitis of the foot. The patient was a male native of Sardinia, 40 years of age, who was suffering from a swollen ankylosed left foot with recurrent pustules and sinuses of four months duration. At the age of 13 years he received a knife wound in the foot. Twenty years later he twisted the foot and was confined to bed a month. From that time on the foot alternately swelled and regressed and the ankle gradually became ankylosed, but no sinuses appeared until four months before operation. The foot was amputated and the patient recovered without incident. The fungus isolated from the tissues of the amputated foot occurred as groups of soft, gray-red granules the size of grains of sand. Although it was cultured and studied, its true nature was not appreciated until two years later when Radaeli<sup>5</sup> isolated and studied an identical fungus from a case of chronic osteomyelitis of the foot, of four years duration in a male Italian. In this case the foot was greatly swollen and deformed in the anterior two-thirds. Recurrent brown to wine-colored swellings and sinuses discharging yellow grains had been present for two years, widely scattered over this area. X-ray showed overproduction of bone in the vicinity of the metatarsals. Despite medical treatment for several months the condition gradually extended to further bone and soft tissue destruction, and painful crises developed which kept the patient in bed for several months at a time. Small, soft, yellow grains transferred from the sinuses to various media gave rise in about three days to small, white, cottony, raised colonies 3 to 5 mm. in diameter. These gradually became brown and extended a short distance into the media. The fungus was further characterized by filiform, interwoven, vegetative hyphae and decumbent, rambling, septate conidiophores 2.5 to 3 microns wide with ascending, somewhat attenuated branches bearing single terminal conidiospores. The conidia were continuous, piriform, oblong, sometimes obovate, truncated at the base, 14 by 5.6 microns, sometimes 11 by 5.7 microns, rarely sub-round, and red-yellow in color. Saccardo named this organism *Monosporium apiospermum*.

Linhaeres and de Magalhaes<sup>6</sup> isolated a fungus from two cases of mycetoma in Brazil. Although the organism was cultivated with difficulty it is generally considered identical with *M. apiospermum*.

## MADURA FOOT DUE TO MONOSPORIUM APIOSPERMUM IN A NATIVE AMERICAN \*

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Clinic of Dr. James B. Bigelow, Holyoke, Mass.)*

Mycetomas are defined by Castellani and Chalmers<sup>1</sup> as "all growths and granulations which produce enlargement, deformity, or destruction in any portion of the tissues of man or animals, and which are caused by the invasion of the affected area by fungi belonging to different genera and species, which produce bodies of varying dimensions, colour, and shape, composed of hyphae, and sometimes chlamydospores, embedded in a matrix. These bodies, which are capable of giving rise to mycelial filaments, on germination, are termed 'grains,' and are found either embedded in the pathological tissue forming these growths and granulations, or escaping freely in the discharge therefrom. In addition, eosinophile bodies can usually be seen."

Two kinds of mycetomas are recognized: actinomycosis in which thirteen species of *Actinomyces* are involved, and maduromycosis in which some fifteen species of various other fungi are the etiological agents. It is remarkable that so great a variety of widely different fungi cause lesions which are clinically almost identical. Mycetoma of the foot is commonly spoken of as Madura foot. The disease is endemic in parts of India and Africa and sporadic in the rest of the world. Boyd and Crutchfield<sup>2</sup> and more recently Gammel<sup>3</sup> reviewed the status of Madura foot in the United States. The latter found twenty-two cases reported, eighteen of these caused by *Actinomyces* and four caused by other fungi.

One of the rarest fungi causing mycetoma is that generically known as *Monosporium*, also referred to as *Scedosporium*. Reports of seven cases of infection with this fungus were found in the literature.

\* Received for publication December 1, 1929.

age. The various tissues in the foot were involved, and black, or red-black, firm grains 1 to 2 mm. in diameter were obtained from the lesion. The grains were composed of densely matted, coarse, yellow-brown filaments and chlamydospores. Although original cultures were obtained with difficulty, subcultures grew readily and the growth remained white. The hyphae were 3 to 4 microns in diameter, septate and gave off a few branches at diverse angles. Older hyphae sometimes measured 5 to 5.5 microns. Fertile hyphae were erect and decumbent, and shorter and wider than vegetative mycelia. Conidiospores were terminal, single, piriform, with truncated base, and measured about 12 by 5 microns. Under unfavorable conditions sclerotia were formed which were considered analogous to the grains formed in the tissues of the host. This is *M. sclerotiale* and differs from *M. apiospermum* in the black color of the grains, slow-growing white colonies, precocious formation of sclerotia, and short, wide fertile branches.

Twenty-six species of *Monosporium* are listed by Saccardo<sup>10</sup> and two others are listed by Farlow<sup>11</sup>; none of these, however, resembles the pathogenic forms described in this paper. In 1911 Saccardo suggested the generic name *Scedosporium* to include the pathogenic species *apiospermum* and *sclerotiale* but no definite evidence could be found that the genus was officially erected.

The case of Madura foot described below is apparently due to *Monosporium apiospermum*.

#### REPORT OF CASE

A native white American male, aged 33 years, applied in December, 1928, for treatment of recurrent swelling and ulceration of the right foot of about ten years duration. He has always lived in Holyoke, Massachusetts, with the exception of eight months in 1920 and 1921 when he was stationed with an artillery unit of the United States Army in the state of Oklahoma, and he has never been outside of the United States. About ten years ago the patient had a lesion over the second metatarsal bone of the right foot which he thought was an injury received while playing football. This was in the nature of an inflammatory process with the slow formation of pustules which opened to the surface, discharged reddish yellow pus, and then healed promptly. While in Oklahoma the patient frequently went swimming barefooted and at this time identical

Montpellier and Gouillon<sup>7</sup> report the case of a male Algerian, aged 35 years, who suffered from a chronic osteomyelitis of the left foot following an injury to the plantar surface four years before. The lesion was characterized by the gradual formation of painless swellings which ulcerated and discharged seropurulent material and pale yellow granules. X-ray showed rarefaction of bone with dislocation of the tarsometatarsal joints. The grains were 0.1 to 1 mm. in diameter and sometimes aggregated. A crushed grain showed mycelial segments 2 to 6 microns long, and egg-shaped spores 10 microns in diameter. Cultures were easily obtained on all moist media and, in addition to the usual form of growth, a slower, wrinkled, mottled brown, scantier and less downy type sometimes occurred without obvious reason. Both types of culture grew the same in hanging drop. A three-day-old culture showed radiating septate mycelia 2 to 4 microns in diameter with usually alternate branches at wide angles. The oldest hyphae tended to be moniliform. Granules were irregularly distributed through the protoplasm. Numerous terminal spores were present. On the fifth day the moniliform character was prominent and the protoplasm was condensed into two or three (usually two) masses resembling nuclei in each cell. On the eighth day many of the segments showed subdivision, isolating two oidia, each containing a nucleus. The spores were increased in number with the age of the culture and depended on oxygen for their formation. They were slightly egg-shaped, 6 to 10 microns in diameter, and terminal or lateral in position. These authors consider the organism identical with that reported by Radaeli but believe it to be an *Aleurisma* and not a *Monosporium*.

Fonseca and de Arêa Leão<sup>8</sup> studied cultures of this organism which they isolated from a patient with yellow-grain mycetoma. In addition to confirming the cultural findings of others they observed the formation of sclerotia in fluid media and in the very moist portions of solid media. Two other forms of reproduction were also described: crescentic terminal single spores attached to the hypha by one end of the crescent, and abundant terminal or intercalary chlamydospores with thickened walls and frequent characteristic small protuberances.

The only other reference found of infection by a fungus of the genus *Monosporium* is that of Pepere<sup>9</sup> who studied a case of Madura foot of eighteen years duration in an Italian peasant 33 years of

presence of the fungus. The fungus masses are round, lobulated or umbilicated. Stained with hematoxylin and eosin, a section of a typical lobulated grain 0.5 mm. in diameter presents an outer bright red zone 0.02 mm. thick which shades into a colorless zone of the same thickness. In both of these portions of the grain, closely packed round bodies about twice the size of red blood corpuscles, and a few hyphae can be made out. The details of these structures are less distinct toward the middle where the colorless zone merges with an amorphous blue or indifferently staining mass making up most of the granule. The faint outlines of a few hyphae can be made out in the central mass. A section of the same granule stained by the Gram-Weigert method (Fig. 3) shows a narrow outer Gram-positive zone of radiating, occasionally branching, coarse hyphae with numerous intercalary and terminal swellings (chlamydospores), and a central Gram-negative amorphous mass in which there are a few Gram-positive hyphae. Although no isolated organisms occur in the tissue, a few faint-staining hyphae project from the margin of the granule.

#### BIOLOGICAL CHARACTERISTICS AND PATHOGENICITY

Cultures of the fungus were made one month after the biopsy, at which time there were two small sinuses in the dorsum of the foot at the site of the biopsy incisions, and a third sinus on the instep. A small amount of sanguinopurulent discharge was present and about twelve light yellow, firm granules up to 1 mm. in diameter were removed from two sinuses. The granules were washed in plain broth, planted on a variety of media, and incubated at 37° C. and at room temperature. Two months later more grains were obtained from one of the sinuses. These gave the same results on cultivation.

The growth is the same on plain agar, serum agar, Sabouraud's agar, blood agar, and dextrose agar, and in initial cultures occurs only where a grain is deposited. Growth is rapid at 37° C. and slower at room temperature. After two days incubation at 37° C. a round, white, cottony mass of hyphae surrounds the granule. Growth proceeds laterally and in four days the center becomes gray-brown. The growth progresses with a narrow, white, advancing margin and eventually the surface of the medium becomes covered with a black surface layer, a velvety brown aerial layer which extends at most 4 mm. above the surface, and a few colorless hyphae extending to an

lesions appeared near the same site and went through the same process of discharging pus and then healing. Since then about twelve similar lesions have appeared from time to time at various sites on the right foot and ankle, and for some years the patient has noticed small yellow grains in the discharge. A typical lesion begins with a small, localized, non-tender swelling which appears somewhere beneath the skin of the foot. It increases in size for several weeks and the overlying skin becomes involved, turns bluish red and ruptures at the central point. The sinus thus formed discharges thin serous fluid, more or less pus, and numerous firm yellow grains up to 1 mm. in diameter. After a period varying from several days to several weeks, the sinus closes leaving a superficial depressed scar. The process has not extended to the toes or to the fibula and tibia. The foot has increased slightly in size and X-ray plates show an overproduction of bone in relation to the os calcis, cuboid and second metatarsal bones. The foot has not prevented the patient from walking about and working until about a year ago when for periods of several days to two weeks there has been severe pain, especially when bearing weight on the foot. The general health is good. Wassermann and Kahn tests of blood taken March 21, 1929 were negative. Red and white blood cell counts on the same date were within normal limits. Although the patient frequently sought medical aid, no form of treatment, including fairly large amounts of iodides, influenced the progress of the disease, and apparently the correct diagnosis was not made until tissue removed December 20, 1928 was examined microscopically. Amputation has been advised, but is refused by the patient. Fig. 1 shows the appearance of the foot in January, 1929.

#### MICROSCOPIC EXAMINATION

The tissue shows small irregular islands of new bone embedded in granulation tissue which is markedly infiltrated with lymphocytes, plasma cells and numerous endothelial leucocytes. Here and there are masses of polymorphonuclear leucocytes, usually with centrally located fungus grains up to 0.5 mm. in diameter (Fig. 2). Each small abscess is surrounded by a zone of endothelial leucocytes, some of which have phagocytosed fragments of other cells. No foreign body giant cells occur in the sections studied. The lesion is a pronounced osteomyelitis which is unusual only because of the

spore contains several round refractile bodies similar to those in the hyphae. Conidiospores germinate promptly when subcultured. Within twelve hours at 37° C. a hypha sprouts from the side or small end (Text-Fig. 1 (b)) and growth proceeds as in original cultures. Germination was never observed in spores previous to separation from the conidiophore. With age the hyphae become more closely segmented, irregular in outline, and frequently form terminal or intercalary swellings up to 10 microns in diameter (Text-Fig. 1 (c)). These are interpreted as chlamydospores. Chlamydospore formation is seen only on and beneath the surface of the medium while conidiospores are on the surface, aerial, and occasionally beneath the surface. Among the aerial hyphae of an eighteen-day culture on Sabouraud's medium there are a few round bodies up to 56 microns in diameter interpreted as sclerotia (Figs. 4 and 5). These are shells of densely interwoven hyphae and contain a substance of indefinite structure which stains poorly.

A grain in nutrient broth incubated at 37° C. develops in a week into a small white cotton-ball colony in the bottom of the tube but does not form spores and the supernatant medium remains clear. Subculture to the surface of broth media gives rise to a felted white growth which remains on the surface and eventually turns brown with the formation of conidiospores.

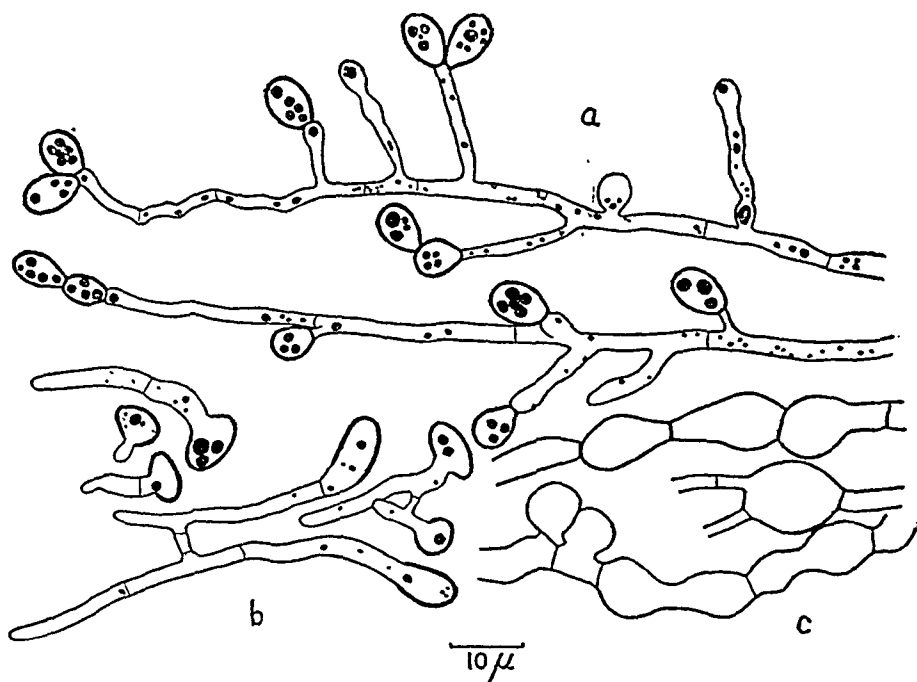
Blood serum and egg medium are liquefied. In the lower, very moist portion of the medium the growth takes the form of a shining, black, wrinkled, tough membrane composed of densely packed hyphae and conidiospores of twice the usual length.

Gelatin is not liquefied. Dextrose and lactose are fermented with the formation of gas. Neither acid nor gas is formed from saccharose.

Two white mice were inoculated intraperitoneally with grains obtained directly from the lesions, and two other white mice received intraperitoneal injections of a three-weeks-old broth culture containing many spores. The animals were observed for one month and no ill effects were noted. No lesions or fungus were found at autopsy. A guinea pig injected with a young broth culture containing hyphae and spores remained well during one month of observation. An old culture containing many spores and hyphae was injected into the foot of another guinea pig without result.

equal depth into the medium. The organism does not grow when grains are inoculated beneath the surface of solid media.

Young hyphae in the white margin of growth are filiform, straight or slightly curved, septate, branch frequently at an angle of 30 to 90 degrees, and occasionally anastomose. The diameter averages 2.9 microns. The only other visible structures in the hyphae are a few round refractile bodies up to 2 microns in diameter scattered



TEXT-FIGURE 1

Growth of *Monosporium apiospermum* on Sabouraud's agar at 37.5° C.

(a) Conidiospore formation, 5 days incubation.

(b) Sprouting conidiospores, 18 hours incubation.

(c) Chlamydospores, 18 days incubation.

throughout. Older hyphae from the brown portion of the colony show many erect and decumbent spore-bearing branches (conidiophores) which in turn are branched (Text-Fig. 1 (a)). In structure these do not differ essentially from the sterile hyphae. The conidiospores are terminal, usually single, and occasionally in pairs side by side or end to end. Some are borne on short, narrow, straight sterigmata arising perpendicularly from the larger hyphae. The conidiospores are brown, thick-walled, egg-shaped, with the smaller slightly flattened end attached to the conidiophore or sterigma, and measure 9.4 microns long by 4.9 microns in greatest diameter. Each



The only other organism encountered in original cultures was a small Gram-negative bacillus corresponding in appearance to an organism seen in smears from the lesions. It was looked upon as a saprophytic invader without significance.

While the characteristics of *M. apiospermum* have been established piecemeal by the various observers and have varied somewhat, the identity of the fungus which we isolated is shown in Table I.

### DISCUSSION

Although Madura foot is a rare disease in the United States, it should be considered in chronic osteomyelitis of the foot. It is usually easy to find the grains and their presence is pathognomonic, but the identification of the infecting organism requires microscopic and cultural study. Diagnosis by biopsy is impossible unless a grain is included in the section, because the lesion is not histologically characteristic. It may be a simple chronic osteomyelitis, resemble tuberculosis, or occasionally simulate lues. The patient with Madura foot, especially of the yellow-grain variety, should be started on iodine therapy, unless there is some contra-indication for the drug, because of the well known response of *Actinomyces* and *Sporotrichum* infections to iodine. Should the organism prove to be some other type, amputation is the only treatment which promises success.

### SUMMARY

1. A case of Madura foot due to *Monosporium apiospermum* is reported.
2. The literature on pathogenic Monosporia is reviewed.

NOTE: We are grateful to Dr. C. W. Dodge for assistance with the mycology.

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TABLE I

		M. apiospermum	Authors' fungus	M. sclerotiale
Grains	color	yellow	yellow	
	size	0.1 to 3 mm.	up to 1 mm.	1 to 2 mm.
Growth		2 to 12 days	2 days	4 to 5 days
	color	white to brown to black	white to brown to black	remains white
Colony	size	small	large	large
	in tissue	1 to 1.5 $\mu$	1.3 $\mu$	1.2 to 1.5 $\mu$
Hyphae	in culture	2 to 4 $\mu$	2.9 $\mu$	3.4 $\mu$
	in tissue	10	about 14	present
Chlamydospores		decumbent and erect	decumbent and erect	decumbent and erect
	size	2.5 to 3 $\mu$	2.9 $\mu$ and less	shorter and thicker than mycelium
Fertile hyphae	shape	ovoid, truncated	ovoid, truncated	ovoid, truncated
	size	4 to 6 by 10 to 14 $\mu$	4.9 by 9.4 to 18 $\mu$	
Sclerotia		occasional	occasional	numerous

## DESCRIPTION OF PLATES

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### PLATE 72

FIG. 1. Madura foot of 10 years duration due to *Monosporium apiospermum*. Two healing biopsy wounds are situated on the dorsum and below the malleolus. Several spontaneous lesions in various stages of swelling, sinus formation and healing are also evident.

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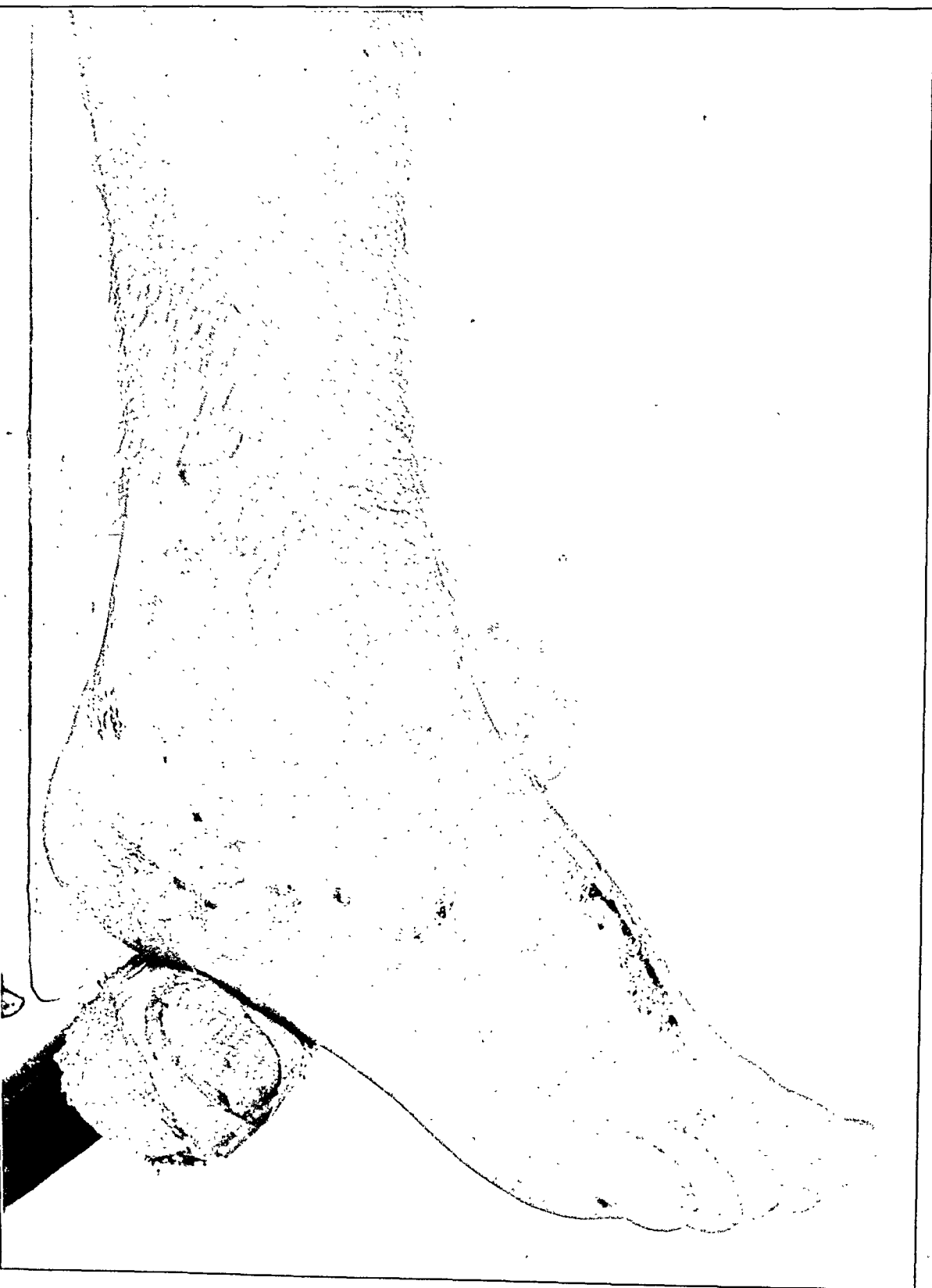
PLATE 73

FIG. 2. Microscopic appearance of the lesion of Madura foot due to *Monosporium apiospermum*. There is a chronic osteomyelitis with an abscess containing a grain of the fungus.  $\times 40$ .

FIG. 3. Section of a grain stained by the Gram-Weigert method. The grain is umbilicated and composed of a central homogeneous mass of dead fungus surrounded by a marginal network of branching hyphae and chlamydospores.  $\times 250$ .

FIG. 4. Section of an eighteen-day culture of *M. apiospermum* on Sabouraud's medium. A growth of hyphae and conidiospores extends into the medium (lower portion of photograph) and for some distance above the surface where conidiospores are most numerous. Four sclerotia are shown.  $\times 200$ .

FIG. 5. Sclerotia. These are composed of dense shells of hyphae.  $\times 750$ .



I

Gay and Bigelow

Madura Foot Due to *Monosporium Apiospermum*

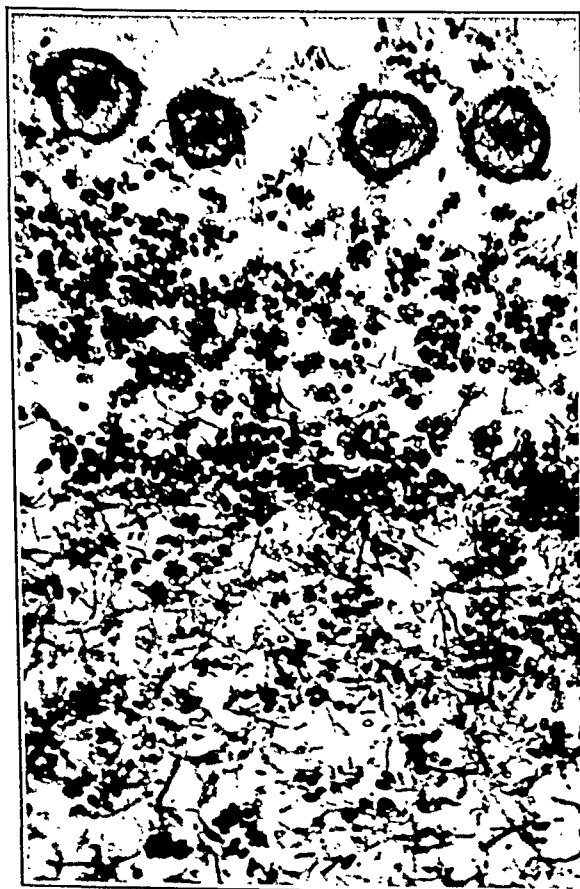




2



3



4



5



cartilages, spleen, ovaries and adrenals. It is worthy of note that calcium deposits were not present in the liver or striated muscle.

In view of the specific manner in which irradiated ergosterol induces calcium deposition in certain tissues while sparing others, it seemed of interest to study its action in certain pathological conditions. The administration of this substance to animals having tuberculosis at once suggested itself, since the caseous portions of tubercles appeared to be especially favorable material for calcification. Rabbits were used because of their great susceptibility to irradiated ergosterol. In order to eliminate the spontaneous calcification that sometimes occurs in chronic tuberculosis of rabbits, a highly virulent strain of bovine tuberculosis was chosen.

Reports of the calcification of the lesions in acute tuberculosis in animals following the administration of irradiated ergosterol have not been found in the literature.

#### MATERIAL AND METHODS

Each of twenty-five young rabbits, averaging 1700 gm. in weight, was given intravenously 1 cc. of a heavy saline suspension of highly virulent bacilli of bovine tuberculosis. Later, a concentrated preparation of activated ergosterol, having one thousand times the antirachitic potency of cod liver oil,\* was administered by stomach tube to each of fourteen of the above rabbits in individual doses of 3 to 5 cc. (Table I). This medication began on the thirteenth day of the experiment with the exception of three rabbits, Nos. 13, 14 and 18, which were started on the seventh, tenth, and tenth day of the experiment, respectively. The administration was continued at frequent intervals (one to three days) until death occurred. The interval between doses and the amount of material per dose was decided individually for each animal after careful consideration of its condition. At all times, the decision was made with a view of prolonging the period of medication rather than of giving large individual doses.

A series of six of the remaining animals were given comparable doses of the inert solvent oil used in the above preparation.

The remaining five rabbits received no medication and served as additional controls.

\* This preparation was furnished through the courtesy of Mead Johnson & Co.

## THE CALCIFICATION OF TUBERCLES BY MEANS OF IRRADIATED ERGOSTEROL \*

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*(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)*

Since the observations of Steenbock<sup>1</sup> and Hess<sup>2</sup> that certain substances acquire antirachitic properties following exposure to ultraviolet light, there has been a great deal of interest centered on the artificial production of vitamin D and on its application to the diseases concerned with calcium metabolism. The production of a potent antirachitic substance by the activation of cholesterol initiated careful chemical studies of this substance. In 1926 Rosenheim and Webster<sup>3</sup> demonstrated that ergosterol acquires remarkable antirachitic power following activation, and that cholesterol loses activatability on purification. Heilbron, Kamm and Morton,<sup>4</sup> Pohl,<sup>5</sup> and Windaus and Hess<sup>6</sup> could identify by spectroscopic study three absorption bands found in cholesterol as characteristic of the vitamin precursor. Bills and his co-workers<sup>7</sup> discovered a fourth absorption band identified with the provitamin, and were further able to show by a comparison of destruction rates that the activatable contaminant of cholesterol was ergosterol. As a result of all these observations it became generally accepted that the specific substance activated by irradiation was ergosterol, a sterol widely distributed in nature.

The efficacy of this substance in the treatment of rickets and osteomalacia has been established. In addition, a few workers (Pfannenstiel,<sup>8</sup> Kreitmair and Moll,<sup>9</sup> Klein,<sup>10</sup> and Smith and Elvove<sup>11</sup>) have studied the effects of massive doses of this material on normal animals. They have found an elevation of the blood calcium followed by calcium deposition in some of the tissues. In a similar study (to be published later) we have found that there is usually a retention of nitrogenous products in the blood before death. The animals showed extensive calcium deposits in the arterial walls, lungs, kidneys, stomach wall, heart muscle, bronchial

\* This study has been aided by a contribution from Mr. William A. Parker.  
Received for publication December 1, 1929.

## PATHOLOGICAL DESCRIPTION

*Lungs:* The pleural surfaces were roughened by innumerable small, rounded, grayish yellow nodules (tubercles) averaging 0.2 cm. in diameter. The cut surface of the parenchyma was studded with closely approximated, rounded lesions (tubercles) of similar size. Occasionally a creamy white exudate filled the larger bronchi. The pulmonary vessels were negative.

*Kidneys:* The kidneys were of normal size and shape. Over the surface were irregularly scattered small grayish white nodules (tubercles). Similar lesions were found within the parenchyma. Pelves, ureters and vessels were negative.

*Aorta:* Two of the fourteen animals which received irradiated ergosterol (Nos. 14 and 18) showed considerable changes in the upper portion of the aorta. Both had a moderate degree of dilatation of the ascending aorta and the arch. A sclerotic process characterized by the formation of irregular depressed lesions of the wall was very evident from the external surface. The intimal surface was roughened by depressions irregular in size and shape, ranging from 0.4 cm. to 0.1 cm. in diameter. The largest lesions were found in the upper portion of the thoracic aorta where only minute partitions separated their adjacent edges. The number and size of these lesions progressively decreased as the bifurcation of the iliacs was approached. The lining of the depressions was firm, and no evidence of ulceration was seen. The elasticity appeared fairly normal. A faint grayish white line (calcium) was seen in the media. Three of the remaining animals showed very slight roughening of the intimal surfaces of the aorta. The remaining nine animals showed no demonstrable gross lesions of the aortae.

The aortae of the eleven control animals were normal.

## MICROSCOPIC DESCRIPTION. FORMALIN FIXATION

*Lungs:* Many large and small areas of calcification were contained in the caseous and necrotic centers of otherwise typical tubercles (Figs. 1 and 2). The deposits were irregular in shape and ranged in size from 0.2 cm. to 0.01 cm. In contrast, the tubercles without necrotic centers rarely contained even a minute trace of deposited calcium. Enmeshed within the calcified centers of the degenerated tubercles were many necrotic cells, acid-fast bacilli and

All animals were allowed to die. Sections from the organs were fixed in an alcohol-formalin mixture (9 parts of 95 per cent alcohol to 1 part of 40 per cent formalin), in 10 per cent formalin, in 95 per cent alcohol, and in Zenker's fluid. Some of the tissues fixed in formalin were embedded in celloidin and later stained either with hematoxylin and eosin or by the silver method of von Kossa. In order to study the relationship of the tubercle bacilli to the calcified deposits it was found necessary in staining the tubercle bacilli to avoid the use of acid alcohol which dissolved out the calcium. Therefore, sections stained with carbol fuchsin were cleared in aniline oil following the method of Flexner for staining the bacillus of leprosy. In order to demonstrate fat, frozen sections of formalin-fixed material were stained with Sudan IV. The tissues fixed in Zenker's fluid were embedded in paraffin and stained with eosin-methylene blue, and by the usual method for the demonstration of tubercle bacilli in sections. The calcium deposits were identified by their solubility in acid and by the following histological criteria, using formalin-fixed material: (1) when stained with hematoxylin and eosin the precipitated calcium appeared as a dark blue, coarsely granular material; (2) after treatment with a silver nitrate solution and counterstaining with 0.5 per cent basic fuchsin the deposits assumed a deep brownish black color.

#### OBSERVATIONS

After a period of approximately ten days, all the animals began to lose weight rapidly and died before the twenty-fourth day of the experiment. Diarrhea played a part in causing the death of rabbits No. 7, 8, 9, 11 and 13, soon after receiving their initial dose of irradiated ergosterol.

While all the organs were examined grossly and microscopically in every animal, only the changes in the lungs, kidneys and aorta are regarded as sufficiently significant to warrant their description in this report. Since macroscopically the lungs and kidneys of the animals which received irradiated ergosterol appeared identical with those of the controls, it is thought that one description will suffice for both. On the other hand, the aortae of the two groups will be described separately as some of the aortae from the animals which were given irradiated ergosterol showed definite macroscopic changes which were not present in those of the control animals.

The kidneys of the eleven control animals contained similar tubercles but did not contain precipitated calcium.

*Aorta:* The aortae of five of the animals which received activated ergosterol contained calcium deposits within the media. In every instance they were limited to the internal elastic lamina and adjacent portion of the media. In rabbits No. 14 and 18 the process was extensive and formed an irregularly thickened calcific ring which involved almost the entire width of the media. In rabbits No. 1, 22 and 23 the deposits of calcium were less extensive and had not fused to encircle the lumen. The calcified areas were irregular in size and shape, and their adjacent edges were separated by normal tissue. The remaining nine animals did not exhibit calcification of the aorta.

The aortae from the eleven control animals were normal.

#### ZENKER'S FIXATION

Calcification was absent in all tissues fixed in Zenker's fluid because the acetic acid had removed the precipitated calcium from the tissues.

*Lungs:* The tubercles of the lungs appeared identical in the animals which received irradiated ergosterol and in the control animals.

*Kidneys:* The tubercles were identical in all animals. Occasionally a faint bluish zone, where calcium had been removed, could be seen in the arteries of some of the rabbits showing the most marked calcification in the formalin-fixed preparations. The cells in this region appeared essentially normal.

*Aorta:* An irregular dark blue-staining rim of tissue in which nuclei and cell boundaries were easily recognized occupied the location of the calcium deposits in the region of the internal elastic lamina. The remaining portions of the wall were quite normal. A slight but similar change was present in the animals showing less calcification. The remainder of the aortae in this series were negative.

The aortae of the controls were negative.

#### DISCUSSION

It has been shown in the experiments reported here that marked calcification of the caseous and necrotic centers of the tubercles of the lung can be produced by the administration of large doses of

fat globules. The central portions of the tubercles were surrounded by a well demarcated margin of connective tissue cells, lymphocytes and endothelial and polymorphonuclear leucocytes. This zone was free from calcific deposits. In no instance was there an apparent change in size, shape or cellular content of the tubercles following the deposition of calcium. In contrast to the majority of the animals, Nos. 14 and 18 exhibited a marked preponderance of non-caseating tubercles over the caseating type and, as in all the other cases, calcium deposition was limited almost entirely to the degenerated lesions. These two animals also showed considerable calcification of the bronchial cartilages, bronchial epithelium and alveolar epithelium. The alveoli were often filled with a serous exudate containing endothelial and polymorphonuclear leucocytes. Occasionally some of the bronchi were filled with an acute inflammatory exudate. Calcification was absent within the vessel walls.

Calcium deposits were absent within the lungs of the five animals which received only one dose of activated ergosterol as well as in the eleven control animals, despite the fact that all contained many caseous and necrotic tubercles.

*Kidneys:* Seven of the animals which received activated ergosterol exhibited calcification in the kidneys. The deposits were prominent in rabbits No. 14 and 18 where both the tubercles and the renal arteries and tubules were conspicuously involved. The kidneys of rabbits No. 2, 20, 22, 23 and 24 contained very scant deposits of calcium which were limited almost entirely to the tubercles and to the walls of the vessels enclosed by them. In general the tubercles in the kidneys were smaller, contained fewer areas of necrosis, and fewer and smaller deposits of calcium than the tuberculous lesions of the lungs. Since the tubercles were of hematogenous origin they were almost always situated around the arteries. The areas containing necrotic cells, polymorphonuclear and endothelial leucocytes were situated nearest to the arterial walls, and exhibited a greater tendency to undergo calcification than the non-necrotic portions of the tubercles. The amount of precipitated calcium was especially prominent in the renal tubules of rabbits No. 14 and 18. It was located in and near the membrana propria, and within casts in the lumina of the tubules. These animals showed large deposits of calcium in the media of the middle-sized and smaller arteries.

activated ergosterol in the last stages of acute tuberculosis in rabbits. That there is a definite tendency for the calcium to precipitate where there is necrosis is shown by the fact that non-necrotic tubercles of the same age do not contain calcium deposits. There is also a tendency for the calcium to precipitate in non-tuberculous locations (kidneys and aorta). However, it has been demonstrated that in some instances large areas of calcification are found in the tubercles with little or no calcification in the normal tissues. It is noteworthy that the five rabbits which received only one dose of irradiated ergosterol did not show any calcium within the tissues.

The dangers of applying these facts to clinical practice are obvious when the toxic manifestations of large doses of irradiated ergosterol are observed in normal animals. We have corroborated the findings of other workers, showing that there occurs a marked loss of weight, cachexia, and calcification of many of the tissues. In addition, we have been able to demonstrate even more widespread calcification than had previously been observed and a retention of nitrogenous products in the blood, associated with marked kidney lesions.

Whether or not these calcium salts would be reabsorbed if the animals lived we are unable to say. It seems likely that other acute inflammatory lesions might also undergo calcification following the use of large doses of irradiated ergosterol.

It should be emphasized that the calcified lesions described in this paper were produced by the use of repeated massive doses of irradiated ergosterol. Single large doses have no such effect.

#### SUMMARY

1. The administration of repeated large doses of activated ergosterol to rabbits suffering from acute tuberculosis causes extensive deposition of calcium salts within the caseous lesions. The administration of a single large dose produces no demonstrable changes.

2. It is suggested that a high vitamin D diet might possibly be useful in some cases of pulmonary tuberculosis.

NOTE: I am indebted to Dr. F. B. Mallory for helpful criticism and for the photomicrographs. I also wish to thank Dr. Frederic Parker, Jr., for his suggestions and criticism.

TABLE I  
*The Effect of Large Doses of Irradiated Ergosterol upon Rabbits with Acute Tuberculosis*

Experiment Number	Duration of medication	Total dose	Number of doses	Died: day of exp.	Initial weight	Calcification of lung	Calcification of kidney	Calcification of aorta
	<i>days</i>	<i>cc.</i>			<i>gm.</i>			
1	10	15	3	23rd	1410	Marked	o	Slight
2	10	15	3	23rd	1370	Marked	Slight calci- fication in vessels with- in tubercles	Slight
7	4	5	1	17th	1800	o	o	o
8	1	5	1	15th	1900	o	o	o
9	3	5	1	17th	1920	o	o	o
11	3	5	1	17th	2020	o	o	o
13	3	5	1	10th	1950	o	o	o
14	8	20	4	15th	2060	Calcification of caseous centers and of alveolar and bronchial epithelium	Considerable	Considerable
18	8	15	3	18th	2100	Calcification of caseous centers and of alveolar and bronchial epithelium	Considerable	Considerable
20	7	15	4	20th	1700	Marked	Slight calci- fication only in tubercles	o
21	4	6	2	16th	1740	Marked	o	o
22	8	14	4	21st	1900	Marked	Very slight	Slight
23	8	14	4	20th	1650	Marked	Slight in tubercles	o
24	7	11	3	19th	1720	Marked	Slight in tubercles	o



## DESCRIPTION OF PLATES

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### PLATE 74

FIG. 1. Section from the lung. Photomicrograph shows calcification of a caseous center of a tubercle.  $\times 100$ .

FIG. 2. Section from the lung. Photomicrograph shows calcium deposition within the caseous portions of closely grouped tubercles.  $\times 100$ .

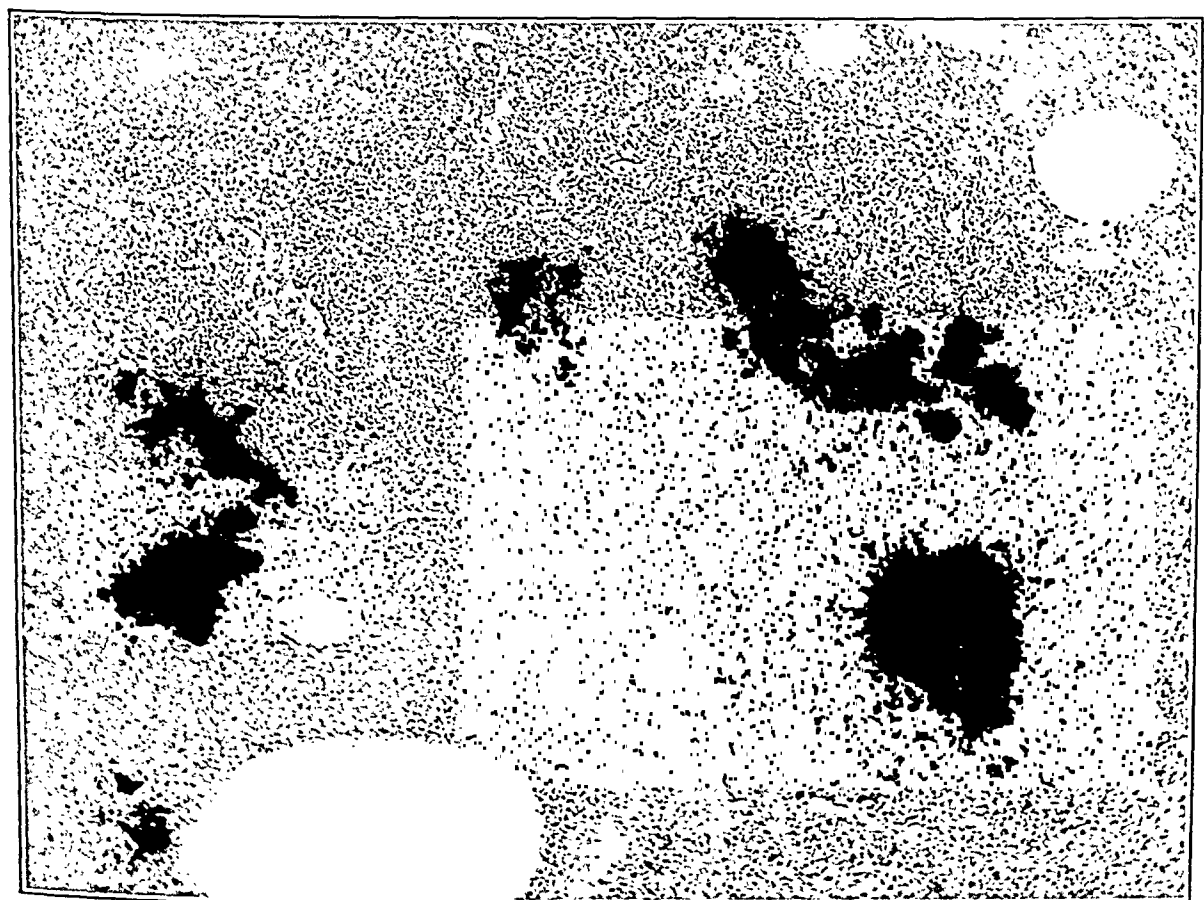
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I



2

Spies

Calcification of Tubercles

Clawson,<sup>19</sup> and Klemperer and Otani<sup>20</sup> has shown that the most conspicuous renal lesions are an arteriosclerosis (a formation of hyalin under the endothelium of the afferent glomerular arterioles) and a hyperplastic elastic tissue intimal thickening of the walls of the small and medium-sized arteries.

In addition Löhlein described a dilatation of the afferent arteriole at its entrance to the glomerular tuft. This finding was confirmed by Fishberg and by Jaffé. Löhlein also observed hyaline areas in the glomeruli continuous with the hyalin of the arterioles. Jaffé suggested that the primary renal lesion be sought in the glomerular tuft where he noted a thickening and hyaline degeneration of the capillary walls.

Volhard and Fahr saw inflammatory glomerular changes in cases of essential hypertension with death from uremia. They believed that the inflammation superimposed on the arteriosclerosis caused the renal insufficiency. Löhlein, Jores and Herxheimer opposed this theory because they thought the glomerular damage was vascular in origin and not inflammatory. More recently Fahr described an acute arteritis of the afferent glomerular vessels as the cause of uremia. Volhard has lately explained the renal insufficiency on the basis of a general vasoconstriction which includes the glomeruli. Fishberg believed the glomerular lesions were non-inflammatory and that uremia was usually due to an acceleration of the arteriosclerotic process. Bell and Clawson found that patients with slowly developing uremia had at autopsy a simple glomerular and tubular atrophy from progressive narrowing of small arteries and arterioles, while those with rapidly developing uremia had a necrosis of arteries and arterioles producing small infarcts, infarcted glomeruli and rarely glomerulitis. Klemperer and Otani ascribed the glomerular changes to ischemia rather than to inflammation.

*Technique:* The tissues available had all been fixed in Zenker's fluid. The stain which proved most valuable was the Mallory-Heidenhain azan carmine.<sup>21</sup> Unless otherwise stated descriptions of glomeruli are made from this stain. Nuclei are red and all connective tissues including glomerular, capsular and tubular basement membranes are blue. Phosphotungstic acid hematoxylin, Weigert and Verhoeff's elastic tissue stains were used on some of the tissues.

*Method of Studying Glomeruli:* For practical purposes there are only three types of glomeruli seen in the primary hypertensive

# HISTOLOGICAL CHANGES IN THE RENAL GLOMERULUS IN ESSENTIAL (PRIMARY) HYPERTENSION \*

## A STUDY OF FIFTY-ONE CASES

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Throughout this paper the term essential (primary) hypertension is used to include all chronic hypertension of unknown etiology. Although this is probably not a homogeneous group, most of the cases are similar clinically and pathologically. They are all characterized by (1) a persistent systolic blood pressure of 150 mm. or more, and (2) a definite left ventricular hypertrophy not associated with any of the diseases known to cause hypertrophy.

The object of this paper is to describe the histological picture of the renal glomerulus in essential hypertension.

The material consists of all the 1927 and 1928 Boston City Hospital autopsy cases which have a history of chronic high blood pressure. After excluding the cases of secondary hypertension due to glomerulonephritis, toxemia of pregnancy, urinary obstruction, nephrosis and aortic insufficiency, there remain fifty-one cases of primary hypertension. The series is subdivided according to the cause of death into (1) a cerebral group (apoplexy) of 16 cases, (2) a cardiac group (myocardial insufficiency and coronary disease) of 19 cases, (3) a renal group (uremia) of 14 cases, and (4) a miscellaneous group (rupture of aorta and diabetes) of 2 cases. The controls are kidneys from nine persons who died in the fifth, sixth, seventh and eighth decades and who were known to have had a normal blood pressure. The object was to determine the glomerular lesions due to age alone.

Before proceeding to a study of these cases, the general conception of the histological changes in the kidney in primary hypertension must be summarized. The work of Johnson,<sup>1</sup> Gull and Sutton,<sup>2</sup> Ziegler,<sup>3</sup> Jores,<sup>4</sup> Herxheimer,<sup>5</sup> Gaskell,<sup>6</sup> Volhard and Fahr,<sup>7, 8, 9, 10, 11</sup> Lohlein,<sup>12, 13, 14</sup> Evans,<sup>15</sup> Fishberg,<sup>16, 17</sup> Jaffe,<sup>18</sup> Bell and

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epithelium around the loop, as well as the adjacent capsular epithelium, swells, proliferates and often undergoes hyaline granular degeneration. The lumen of the loop is occluded by fatty swollen endothelium, inflammatory cells, fibrin and hyaline fibers. These fibers, which become collagen, contract and destroy the function of the loop. The adjacent capsular epithelium may proliferate and form crescents. The lobule with the focal lesion often becomes adherent to the capsule during the inflammatory and healing processes.

Glomeruli with focal inflammation are more numerous than those with diffuse involvement. The cerebral group of hypertensives shows the fewest inflammatory lesions while the renal group shows the highest. Every uremic death in this series is associated with inflammatory glomeruli.

*The Hypertensive Contracted Glomerulus:* (Figs. 2 and 4.) This glomerulus is always smaller than normal, its diameter often being reduced one-half. The decrease in size of the glomerulus produces an apparent increase in the pericapsular connective tissue and gives it a lamellated appearance. The capsular basement membrane is thickened. Its epithelial lining is normal.

The normal glomerular tuft has multiple loops opening out of the main lobules. In a hypertensive glomerulus the original lobulation remains but the loops are decreased in number. This gives the glomerulus a simpler structure. Glomerular epithelium and endothelium are unchanged. The striking feature is a uniform thickening and wrinkling of the glomerular basement membrane. The process seems to proceed diffusely throughout the tuft. Normally the basement membrane is thin and not wrinkled. All transitions from normal are present in one section. In this study only glomeruli in advanced stages of the lesion are counted as hypertensive.

There is no intracapillary impediment to the circulation in such a glomerulus. The afferent arteriole, the capillaries of the tuft and the efferent vessel are always open. The afferent arteriole is not dilated or narrowed at its entrance to the glomerulus.

Occasionally a few of these glomeruli show focal and diffuse inflammatory lesions. The result is occlusion of the whole tuft or part of it and eventual hyalinization. The afferent arteriole usually shows lesions at the hilum of an inflammatory glomerulus.

The percentage of hypertensive contracted glomeruli is 47 in the renal and miscellaneous groups, 33 in the cardiac group, 24 in the cerebral group and 0.8 in the control group (Table VI).

kidney: (1) non-hypertensive glomeruli, some of which may show diffuse or focal inflammatory lesions, (2) hypertensive contracted glomeruli, a few of which may also contain diffuse or focal inflammatory areas, and (3) hyaline glomeruli.

One hundred consecutive glomeruli were counted in each section and classified as (1), (2) or (3). Subheadings under non-hypertensive and under contracted hypertensive were used to indicate the number of such glomeruli showing focal or diffuse inflammatory reactions.

*Non-Hypertensive Glomeruli:* This term is used to include (1) all normal glomeruli, and (2) all glomeruli which are normal except for focal or diffuse inflammatory lesions.

The reader is referred to a previous paper by the author<sup>21</sup> for a description of the histology of the normal glomerulus. Briefly, the glomerular epithelium and basement membrane are continuous with the tubular epithelium and basement membranes respectively. Likewise, the endothelium of the glomerulus is a continuation of the endothelium of the afferent and efferent vessels. The wall of the tuft contains three structures, which from within out are endothelium, basement membrane and epithelium. These are easily distinguished from one another with the Mallory-Heidenhain azan carmine stain (Figs. 1 and 3).

As Table VI shows, the average percentage of non-hypertensive glomeruli is 20 in the renal group, 44 in the miscellaneous group, 52 in the cardiac group, 63 in the cerebral group and 96.2 in the control group. In the hypertensive series many of these normal glomeruli are hypertrophied in an attempt to compensate for atrophied units.

The inflammatory lesions which occur may be diffuse and involve the whole tuft, or focal and involve only one or two loops. When the whole glomerulus is affected the lesion seems to be identical with that described by the author<sup>22</sup> for acute and chronic glomerulonephritis. However, only a very small number of glomeruli are involved. In early stages the glomerulus is enlarged, the capsular and glomerular epithelium proliferates and the capillary loops are occluded by inflammatory mononuclear cells, swollen endothelium and hyaline fibers (Fig. 5). The latter gradually contract and slowly obliterate the capillary lumen. The end result is a hyaline glomerulus.

Usually only a portion of a tuft is involved. The glomerular



TABLE I  
Essential Hypertension (Cerebral Group)

Case number	Age	Sex	Cause of death	Blood pressure	Known duration of symptoms	Summary of history	Blood urea nitrogen	Blood non-protein nitrogen	Urine phenol sulpho-neph-thalein	Heart weight
	years			mm. mercury			mg. per cent	mg. per cent	per cent	gm.
I	32	f	cerebral hemorrhage	235/125	9 years	9 years ago edema throughout first pregnancy. 5 years ago edema and high blood pressure with second pregnancy. From then on, edema, headaches, dyspnea and nausea. Fundi show exudate and old scars.	..	28	30	370
2	42	m	"	190/120	?	Sudden loss of consciousness.	..	..	..	350
3	43	f	"	270/130	6 months	Not well for past 6 months. 5 days ago became delirious then stuporous.	..	48	..	410
4	48	m	"	200/120	15 years	Cerebral shocks 15 years ago, 5 years ago and 4 days ago. Headaches and high blood pressure for years.	..	..	..	500
5	49	f	"	185/115	?	Sudden loss of consciousness.	..	..	..	400
6	53	f	"	230/130	?	Sudden loss of consciousness.	..	..	..	500
7	55	m	"	230/140	2½ years	Sudden loss of consciousness.	..	..	..	540
8	60	m	"	250/110	several years	High blood pressure for several years. 4 days ago a cerebral accident.	..	..	..	370
9	60	f	"	155/110	20 years	20 years of palpitation and dyspnea on exertion. 5 years of attacks of angina, failing vision and nocturia. 4 days of cyanosis, peripheral edema and coma.	..	..	..	530
10	65	m	"	150/95	?	Admitted comatose, cyanosed with edema of legs one day after cerebral accident.	..	..	..	550
11	68	f	"	245/120	3 years	3 years of treatment for high blood pressure. 2 weeks of weakness, dizziness and headaches. Vessels of fundi tortuous. One day ago a sudden cerebral accident.	..	..	..	420
12	70	f	"	high	3 years	3 years ago had high blood pressure and a cerebral shock. Admitted in coma.	..	..	..	350
13	71	m	"	220/140	10 years	10 years of periodic dyspnea and edema. 2 years ago high blood pressure and a cerebral accident. Admitted in coma.	..	37	..	500

*The Hyaline Glomerulus:* This type of glomerulus is usually the result of atrophy due to sclerosis of arteries or arterioles. Herxheimer has shown that a small percentage is congenital. The others are due to the healing of inflammatory processes. The latter explanation applies particularly to hyalinization of single loops or lobules.

Glomeruli which atrophy following disease of arteries are usually found in wedge-shaped cortical areas. Those which atrophy due to arteriolar disease are often scattered in groups throughout the kidney. All transition stages between hypertensive and hyaline glomeruli are found in one section. Anilin blue stains the thickened glomerular basement membrane in the midst of the hyaline mass.

The average percentage of hyaline glomeruli is 33 in the renal group, 8 in the miscellaneous group, 15 in the cardiac group, 13 in the cerebral group and 4 in the control group.

*Renal Vessels:* In this paper the term arteriole is used to indicate the afferent and efferent glomerular vessels, the former arising from the interlobular artery. Serial sections show that the efferent arteriole is normal. The condition of the afferent vessel varies along its course. A distal segment two to three hundred microns long, adjacent to the glomerulus is usually normal. The proximal portion of the arteriole may show an irregular subendothelial fatty hyaline deposit which narrows the lumen. Elastic tissue stains prove that such homogeneous material is internal to the elastic membrane. This type of arteriole may supply (1) an apparently normal glomerulus, or (2) an early hypertensive glomerulus, or (3) a typical hypertensive contracted glomerulus. The arteriolar lesion must then precede or be independent of the glomerular lesion. However, the serial sections show that every hypertensive glomerulus has an afferent vessel which is definitely narrowed and sclerosed in some portion of its course, usually at some distance from the hilum of the tuft. The arteriolar change must, therefore, be related to the basement membrane thickening and wrinkling. The lesions are similar in that the hyaline thickening is subendothelial in both arteriole and glomerulus. Apparently the arteriolar change takes place first.

A dilatation of the afferent arteriole at the entrance to the glomerulus in early cases of hypertension was described by Jaffé and Löhlein. According to my study all normal glomeruli in the control series show this dilatation and only the normal glomeruli in the hypertensive series.

TABLE I (continued)

Case number	Age	Sex	Cause of death	Blood pressure	Known duration of symptoms	Summary of history	Blood urea nitrogen	Blood non-protein nitrogen	Urine phenol sulpho-neph-thalein	Heart weight
14	years 72	m	cerebral hemorrhage	mm. mercury 180/90	15 years	15 years ago a cerebral accident. Two more while in hospital with erysipelas.	mg. per cent ..	mg. per cent 42.5	per cent ..	gm. 350
15	77	m	"	220/115	?	19 days ago a cerebral accident. Coma and hemiplegia since then.	..	..	..	390
16	80	f	"	210/100	1 year	1 year of high blood pressure, dizziness, nocturia and dyspnea.	..	..	..	320

TABLE II

*Essential Hypertension (Cardiac Group)*

Case number	Age	Sex	Cause of death	Blood pressure	Known duration of symptoms	Summary of history	Blood urea nitrogen	Blood non-protein nitrogen	Urine phenol sulpho-neph-thalein	Heart weight
17	years 39	m	cardiac insufficiency	mm. mercury 190/130	1 month	1 month of dyspnea, edema, nocturia and ascites. Decomensation.	mg. per cent ..	mg. per cent ..	per cent ..	gm. 660
18	39	f	"	210/170	7 years	7 years of high blood pressure. 1 month of dyspnea, cough, cyanosis and edema. Decomensation.	..	60	..	580
19	50	m	"	175/105	1 year	1 year of angina pectoris, dyspnea and pleural effusion. Decomensation.	..	..	..	760
20	54	m	"	162/120	1 year	1 year of high blood pressure, dyspnea, cough, edema and nocturia. Decomensation.	..	..	..	860
21	55	m	"	240/160	2 years	2 years of nocturia, dyspnea weakness and epistaxis. 6 weeks of palpitation and edema. Decomensation.	..	50	..	560
22	55	f	"	240/130	3 years	3 years of headache, blurred vision, palpitation, praecordial pain, edema and nocturia. Decomensation.	23.30	..	..	500
23	56	m	"	160/115	5 years	5 years of cough and epistaxis. 3 weeks of edema and dyspnea. Decomensation.	..	..	..	560

TABLE I  
*Essential Hypertension (Cerebral Group)*

Coronary sclerosis	Mural thrombosis	Weight of kidneys	Sclerosis small and medium-sized renal arteries	Sclerosis of renal afferent arterioles	Tubular atrophy	Glomeruli						
						Non-Hypertensive			Hypertensive contracted			Hyaline
						No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
-	absent	gm. 210	+++	++++	++	per cent 44	per cent ..	per cent ..	per cent 30	per cent ..	per cent ..	per cent 26
-	absent	158	++	++	+	55	..	..	34	2	..	9
-	absent	310	++	++++	++	62	..	..	30	..	..	8
++++	present	240	+	+	-	80	..	..	15	..	..	5
+	absent	300	+	+	-	70	..	..	22	..	..	8
++	absent	200	+	++	+	77	..	..	21	..	..	2
-	absent	280	++++	+++	++++	27	..	..	29	..	1	43
-	absent	210	+++	+++	++	48	..	..	40	..	..	12
++++	present	270	+	+	++	34	..	..	30	2	..	34
-	absent	250	+	++	++	57	2	3	20	..	..	18
+++	absent	320	++	+++	++	62	11	..	22	..	..	5
++++	absent	200	++	+	-	75	..	..	20	..	..	5
++++	absent	240	++	+	++	77	..	..	13	..	..	10

TABLE II (continued)

Case number	Age	Sex	Cause of death	Blood pressure	Known duration of symptoms	Summary of history	Blood urea nitrogen	Blood non-protein nitrogen	Urine phenol sulpho-nephthalein	Heart weight
	years			mm. mercury			mg. per cent	mg. per cent	per cent	per cent
24	56	m	cardiac insufficiency	180/120	12 years	12 years ago two cerebral accidents. At that time, blood pressure 128/80, dizziness, dyspnea, edema and nocturia. 4 years ago blood pressure 170/80, headaches, nocturia, edema and ascites. Decompensation.	..	..	35	830
25	57	f	"	150/60	4 months	4 months of dyspnea, edema and weakness. Decompensation.	..	..	..	500
26	58	f	"	252/148	3 years	3 years of dyspnea and high blood pressure. 4 months of decompensation.	..	50	..	560
27	63	m	"	200/140	11 months	11 months of decompensation.	..	..	..	850
28	63	m	"	210/110	2 years	2 years of high blood pressure. 2 years ago a cerebral accident. Now angina pectoris and decompensation.	..	..	..	515
29	64	m	"	170/110	4 years	4 years of dyspnea, cough, nocturia and edema. Decompensation.	..	..	..	475
30	70	f	"	200/130	18 months	18 months of dyspnea. 1 month of angina pectoris and fibrillation.	..	..	..	520
31	70	f	"	200/?	9 months	9 months of weakness and dyspnea. 2 weeks of ascites and cyanosis. Decompensation.	..	..	..	520
32	74	m	"	140/80	6 years	6 years of dyspnea, edema and angina pectoris. Decompensation.	..	..	..	600
33	80	m	"	170/95	7 weeks	7 weeks of dyspnea, weakness, dizziness and edema. Decompensation.	..	45	..	600
34	80	f	"	160/110	9 years	9 years ago a cerebral accident. 5 years ago another. Decompensation.	..	..	..	400
35	82	m	"	170/110	3 months	3 months of edema and cyanosis. Tortuous retinal arteries. Decompensation.	..	82	..	750

TABLE I (continued)

Coronary sclerosis	Mural thrombosis	Weight of kidneys	Sclerosis small and medium-sized renal arteries	Sclerosis of renal afferent arterioles	Tubular atrophy	Glomeruli						
						Non-Hypertensive			Hypertensive contracted			Hyaline
						No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
—	absent	gm. 320	—	—	—	per cent 65	per cent ..	per cent ..	per cent 15	per cent ..	per cent ..	per cent 20
++	absent	290	++	++	+	75	..	..	15	..	..	10
++	absent	320	—	—	—	91	..	..	4	..	..	5

TABLE II

*Essential Hypertension (Cardiac Group)*

Coronary sclerosis	Mural thrombosis	Weight of kidneys	Sclerosis small and medium-sized renal arteries	Sclerosis of renal afferent arterioles	Tubular atrophy	Glomeruli						
						Non-Hypertensive			Hypertensive contracted			Hyaline
						No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
+++	present	gm. 370	++	+	—	per cent 70	per cent ..	per cent ..	per cent 25	per cent ..	per cent ..	per cent 5
+	absent	290	++++	++++	++	34	1	1	46	2	2	14
+++	present	300	+++	+	+	47	2	4	37	..	..	10
?	absent	520	+	+	—	55	..	..	15	..	..	30
+++	absent	220	++++	++++	++	18	3	2	73	1	..	3
++	absent	270	++	+	+	42	..	..	40	..	..	18
—	absent	270	++	+	—	80	..	..	8	..	..	12

TABLE III  
Essential Hypertension (Renal Group)

Case number	Age	Sex	Cause of death	Blood pressure	Known duration of symptoms	Summary of history	Blood urea nitrogen	Blood non-protein nitrogen	Urine phenol sulpho-naphthalein	Heart weight
	years			mm. mercury			mg. per cent	mg. per cent	per cent	gm.
36	34	f	uremia	280/150	3 years	3 years of headaches, dizziness, blurring of vision and epistaxis. Fundi show old hemorrhages. Uremia.	37.78	..	..	480
37	37	f	"	220/120	1 year	1 year of epistaxis, failing vision, polyuria, dyspnea and palpitation. 3 months of headache, edema and praecordial pain. Fundi show recent and old hemorrhages. Uremia.	58.26	..	0	542
38	46	m	"	227/140	3 months	3 months of praecordial pain, dyspnea dizziness, edema, nocturia and vomiting. Albuminuric retinitis. Uremia.	..	193	0	470
39	47	m	"	245/160	7 years	7 years of headaches, weakness and nocturia. 3 years of high blood pressure and impaired vision. Dyspnea and edema. Uremia.	200.4	..	23	775
40	50	f	"	260/?	3 years	3 years of high blood pressure, dyspnea, headaches and nocturia. 2 years ago a cerebral accident. Uremia.	..	111	..	485
41	50	m	"	high	3 years	3 years of heart trouble. A few weeks of dyspnea, cyanosis, edema and vomiting. Uremia.	..	..	..	640
42	52	m	"	240/150	1 year	1 year of high blood pressure, weakness, dyspnea and epistaxis. Cerebral accident with facial paralysis. Uremia.	..	135	20	570
43	53	m	"	208/126	3 years	3 years of palpitation, epistaxis and nocturia. 6 weeks of cough, dyspnea and praecordial pain. Fundi show exudate and old hemorrhages. Uremia.	135	..	..	520
44	57	m	"	236/144	6 years	6 years of nocturia. 8 months of headache, blurred vision, epistaxis, dyspnea and weakness. Fundi show old hemorrhages. Uremia.	69	..	..	510
45	57	m	"	230/170	3 years	3 years of high blood pressure, dyspnea, praecordial pain, nocturia and edema. Months of chronic uremia.	..	166	0	555
46	58	m	"	230/160	3 years	3 years ago a cerebral accident. Since then, angina pectoris, headaches, dyspnea, edema and chronic uremia. Fundi show new and old hemorrhages.	..	243	0	660
47	60	m	"	208/178	?	Semi-comatose with clinical uremia.	..	..	..	590
48	69	f	"	200/110	6 months	6 months of angina pectoris, weakness, palpitation and dyspnea. Fundi show old hemorrhages. Uremia.	..	300	..	390
49	71	m	"	high	2 weeks	2 weeks of cough, weakness, cyanosis, delirium and twitching. Uremia.	..	75	..	435

TABLE II (continued)

Coronary sclerosis	Mural thrombosis	Weight of kidneys	Sclerosis small and medium-sized renal arteries	Sclerosis of renal afferent arterioles	Tubular atrophy	Glomeruli						
						Non-Hypertensive			Hypertensive contracted			Hyaline
						No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
		gm.				per cent	per cent	per cent	per cent	per cent	per cent	per cent
++++	present	310	+++	++++	+	52	..	..	30	..	..	18
++	absent	185	++++	++++	++++	26	2	10	10	..	..	52
-	absent	small	++++	++++	++++	38	2	1	20	8	1	30
+	absent	410	++	+	+	68	..	..	18	..	..	14
++++	absent	240	++++	++++	++++	10	3	4	66	2	..	15
+	absent	310	+	+	+	57	..	..	23	..	..	20
++++	present	380	+	+	-	72	..	..	13	..	..	15
+++	absent	400	++	+	-	83	..	..	9	..	..	8
++++	absent	320	++	+	-	76	..	..	12	..	..	12
++	absent	400	++	+	-	75	..	..	17	..	..	8
++++	present	400	++	++	-	66	..	..	30	..	..	4
-	absent	290	++++	++	+	35	..	2	48	2	2	11



TABLE IV  
*Essential Hypertension (Miscellaneous Group)*

Case number	Age	Sex	Cause of death	Blood pressure	Known duration of symptoms	Summary of history	Blood urea nitrogen	Blood non-protein nitrogen	Urine phenol sulpho-neph-thalein	Heart weight
50	years 47	m	rupture of aorta	mm. mercury 242/140	6 years	6 years of headaches and dyspnea. 1 year of high blood pressure and edema. 3 days of acute abdominal pain.	mg. per cent ..	mg. per cent ..	per cent ..	gm. 1280
51	56	m	diabetes	208/104	several years	Diabetes for years. Admitted in coma and died in 3 days.	..	33	..	770

TABLE V  
*Control Group (Kidneys from Non-Hypertensives in the 5th, 6th, 7th and 8th Decades)*

Case number	Age	Sex	Cause of death	Blood pressure	Heart weight	Coronary sclerosis	Mural thrombosis	Weight of kidneys
N 1	years 42	m	carcinoma stomach	mm. mercury 120/80	gm. 255	—	absent	gm. 305
N 2	45	f	carcinoma breast	112/76	small	—	"	300
N 3	54	m	pulmonary tuberculosis	normal	340	—	"	480
N 4	55	m	carcinoma esophagus	105/75	260	—	"	380
N 5	58	m	chronic lymphatic leukemia	120/80	300	—	"	360
N 6	60	f	perforated gastric ulcer	normal	260	—	"	280
N 7	60	m	carcinoma esophagus	104/72	290	—	"	275
N 8	70	m	lobar pneumonia	110/80	310	—	"	250
N 9	80	f	mitral stenosis with decompensation	120/90	320	—	"	210

TABLE III  
*Essential Hypertension (Renal Group)*

Coronary sclerosis	Mural thrombosis	Weight of kidneys	Sclerosis small and medium-sized renal arteries	Sclerosis renal afferent arterioles	Tubular atrophy	Glomeruli						
						Non-Hypertensive			Hypertensive contracted			Hyaline
						No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
		gm.				per cent	per cent	per cent	per cent	per cent	per cent	per cent
++	absent	200	++++	+++	+++	25	2	2	62	6	1	2
++	absent	215	++++	++++	++++	46	..	..	66	3	2	23
?	absent	110	++++	++++	++++	..	..	..	27	3	..	70
+	absent	150	++++	++++	++++	4	2	1	47	2	4	40
-	absent	100	++++	++++	++++	10	8	..	44	5	..	33
+	absent	380	++++	+++	++++	20	10	2	7	6	..	55
+++	absent	300	++++	++++	++++	15	2	2	52	8	3	18
+	absent	165	+++	++++	++++	20	4	..	27	1	7	41
++	absent	285	++++	++++	++++	34	..	..	55	4	..	7
?	absent	195	++++	++++	++++	..	..	..	76	4	..	20
+++	absent	220	++++	++++	++++	14	1	..	46	5	1	33
-	absent	150	++++	++++	++++	6	1	..	8	9	3	73
+++	absent	80	++++	++++	++++	20	8	..	18	5	11	38
+	absent	288	++++	++++	+++	20	1	2	64	1	2	10

No one of the fifty-one cases happened to have the acute inflammatory arteritis described by Fahr or the arteriolar necrosis with glomerular infarction seen by Bell and Clawson. Inflammatory

TABLE VI  
*Summary of Glomerular Lesions*

	Number of cases	Average age	Non-Hypertensive glomeruli	Hypertensive contracted glomeruli	Hyaline glomeruli
		years	average per cent	average per cent	average per cent
Non-hypertensive control group	9	58	96.2	0.8	4
Essential hypertension cerebral group	16	59	63	24	13
Essential hypertension cardiac group	19	55	52	33	15
Essential hypertension miscellaneous group	2	51	44	48	8
Essential hypertension renal group	14	52	20	47	33

glomeruli in my series usually have arterioles with a very marked degree of sclerosis or thrombosis extending up to or through the hilum.

Table V shows that there is no arteriolosclerosis in the control group. Two of the cerebral group also show no arteriolosclerosis (Table I, cases 14 and 16) although they have a small percentage of hypertensive glomeruli. This discrepancy may be due to the relative size of glomeruli and arterioles, the former being easier to find than the latter.

The interlobular, small and medium-sized arteries of the kidneys show the changes described by others, namely, intimal atherosclerosis and hyperplastic elastic tissue intimal thickening. As noted in Table V, four of the control series have a mild degree of this sclerosis.

## DISCUSSION

The glomeruli in essential hypertension are usually described as normal. In my series of fifty-one cases the renal group averages only 20 per cent normal glomeruli, the miscellaneous group 44 per cent, the cardiac group 52 per cent and the cerebral group 63 per cent. Nine non-hypertensive controls from the fifth, sixth, seventh and eighth decades average 96.2 per cent normal glomeruli (Figs. 1 and 3).

TABLE IV

*Essential Hypertension (Miscellaneous Group)*

Coronary sclerosis	Mural thrombosis	Weight of kidneys	Sclerosis small and medium-sized renal arteries	Sclerosis of renal afferent arterioles	Tubular atrophy	Glomeruli						
						Non-Hypertensive			Hypertensive contracted			Hyaline
						No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
?	absent	gm. 490	++++	++++	+++	per cent 18	per cent ..	per cent 2	per cent 67	per cent 4	per cent ..	per cent 9
++++	absent	455	++	++	+	68	..	..	22	..	..	10

TABLE V

*Control Group (Kidneys from Non-Hypertensives in the 5th, 6th, 7th and 8th Decades)*

Sclerosis of small and medium-sized renal arteries	Sclerosis of renal afferent arterioles	Tubular atrophy	Glomeruli						
			Non-Hypertensive			Hypertensive contracted			Hyaline
			No inflam.	Focal inflam.	Diffuse inflam.	No inflam.	Focal inflam.	Diffuse inflam.	
-	-	-	per cent 98	per cent ..	per cent ..	per cent ..	per cent ..	per cent ..	per cent 2
-	-	-	95	..	..	..	..	..	5
-	-	-	95	..	..	..	..	..	5
+	-	-	100	..	..	..	..	..	..
-	-	-	94	..	..	3	..	..	3
-	-	-	91	..	..	2	..	..	7
+	-	-	97	..	..	..	..	..	3
+	-	-	92	..	..	2	..	..	6
++	-	-	92	..	..	..	..	..	8

Inflammatory glomeruli (Fig. 5) may occur in all types of essential hypertension and it is not justifiable to separate benign (without renal insufficiency) and malignant (with renal insufficiency) on such lesions. However, the greatest number of inflammatory glomeruli is found in the renal group (Table III) and a high percentage is usually indicative of a uremic death. All cases in the uremic group of this series show glomeruli with inflammatory lesions. The changes in these glomeruli are considered inflammatory rather than vascular in origin because they are generally identical with the intracapillary lesions of glomerulonephritis.

It has been suggested by others that the younger patients dying of essential hypertension have a greater number of inflammatory glomeruli. My series does not support such a view, for Cases 47 and 48, aged 60 and 69 years respectively, have the greatest inflammatory change. Neither is the highest percentage of hypertensive contracted glomeruli found in younger cases. Cases 21 and 49 who were 55 and 71 years of age have the greatest number of such glomeruli. There are not sufficient data available to determine the correlation between duration of symptoms and percentage of hypertensive glomeruli.

The renal group of essential hypertension has much in common clinically and microscopically with those cases of chronic glomerulonephritis which have severe vascular disease. Death is from uremia in both conditions and microscopically both show arteriosclerosis and inflammatory glomeruli. However, in the kidney of chronic glomerulonephritis almost all the glomeruli are diffusely inflamed, while there are rare hypertensive and practically no normal glomeruli. On the other hand, the renal hypertensive kidney has a small number of diffusely inflamed glomeruli, a larger number with focal lesions, a high percentage of hypertensive glomeruli and a definite proportion of normal glomeruli. An anilin blue stain shows that glomerulonephritis is an intracapillary obstruction of the tufts while essential hypertension is a change in the glomerular basement membrane.

### CONCLUSIONS

1. The glomerular lesion of essential hypertension is as typical as the arteriolar lesion. It consists of a decrease in size and a simplification of the glomerulus with a marked thickening and wrinkling of the glomerular basement membrane.

All fifty-one cases show the presence of a typical glomerulus with a thickened wrinkled basement membrane (Figs. 2 and 4). The average percentage of such glomeruli is 48 in the renal and miscellaneous groups, 31 in the cardiac group and 25 in the cerebral group. In all except Case 16 these hypertensive contracted glomeruli are present in sufficient numbers to indicate the diagnosis. It is interesting that this case according to the data available is undoubtedly primary hypertension and yet there is no cardiac hypertrophy and no arteriolosclerosis.

Hypertensive contracted glomeruli are most numerous in kidneys with extensive arteriolosclerosis (Tables I, II, III and IV). Microscopically either lesion may be considered indicative of clinical hypertension. In early cases it is simpler to look for hypertensive glomeruli than for arteriolar disease.

The thickening of the glomerular basement membrane may be the result of a spasm of the tuft or of atrophy due to ischemia or to partial disuse. Serial sections show that sclerosed arterioles may lead to normal glomeruli but that hypertensive glomeruli always have narrowed arterioles. These two facts suggest that the arteriolar lesion precedes and is related to the glomerular change. The portion of the afferent vessel close to the hypertensive glomerulus is usually normal. The subendothelial hyaline deposit with its resultant narrowing of the lumen is some distance from the glomerulus.

There are all transitions from normal to hypertensive hyaline glomeruli.

Bell<sup>23</sup> has described a diffuse thickening of the basement membrane in all the glomeruli in three cases of lipid nephrosis. There was no hypertension, no tubular atrophy and no arteriosclerosis. Evidently a glomerular basement membrane thickening need not be secondary to vascular disease. The glomeruli in such a type of lipid nephrosis are normal in size and lobulation, in contrast to the glomeruli of hypertension which are small with a decreased number of loops. Also, in these three cases of lipid nephrosis the basement membrane of *all* the glomeruli is involved while in hypertension the percentage varies.

The glomerular tuft may be thought of as a filter with the glomerular basement membrane as the gel lying between endothelium and epithelium. The greatly increased thickness of the gel in hypertensive glomeruli must alter its permeability. This change may explain the urinary albumin in such cases.

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## DESCRIPTION OF PLATES

### PLATE 75

FIG. 1. A normal glomerulus from Case 49. Essential hypertension with death from uremia in a man of 71 years. Only 20 per cent of his glomeruli were normal. The photomicrograph shows (1) the normal thinness and continuity of the glomerular basement membrane which forms the middle layer of the wall of the tuft, (2) the continuous layer of glomerular epithelium lying external and adjacent to the membrane, and (3) a few endothelial cells lining the internal surface of the membrane. Stain, Mallory-Heidenhain azan carmine.  $\times 500$ .

FIG. 2. A hypertensive contracted glomerulus from Case 37. Essential hypertension with death from uremia in a woman 37 years of age. Seventy-one per cent of her glomeruli were of this type. The photomicrograph shows (1) a small simplified glomerulus with many less loops than normal, and (2) a marked thickening and wrinkling of the glomerular basement membrane. Stain, Mallory-Heidenhain azan carmine.  $\times 500$ .

In this series of fifty-one cases, the average percentage of such glomeruli is 47 in the renal group (death from uremia), 33 in the cardiac group (death from myocardial insufficiency or coronary disease), and 24 in the cerebral group (death from apoplexy).

2. The arteriolosclerosis precedes and is related to the change in the glomerular basement membrane.

3. Kidneys from individuals dying in the fifth, sixth, seventh and eighth decades with a history of normal blood pressure show 96.2 per cent normal glomeruli. A rare hypertensive contracted glomerulus may be found.

4. There are inflammatory glomeruli in any type of essential hypertension but they are most numerous in the renal group. The lesions are usually focal and as many as 15 per cent of the glomeruli may be involved.

5. Anilin blue (Mallory-Heidenhain azan carmine) is recommended as a routine stain for kidney tissue. It is particularly helpful to differentiate the renal group of essential hypertension from those cases of chronic glomerulonephritis which have extensive vascular disease.

NOTE: I am indebted to Dr. F. B. Mallory for suggestions and criticism and also for the photomicrographs.

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## PLATE 76

FIG. 3. Two normal loops from a normal glomerulus. Case 50. Essential hypertension with death from rupture of the aorta in a man 47 years of age. Only 18 per cent of his glomeruli were normal. The photomicrograph shows (1) the thinness of the normal glomerular basement membrane, and (2) the continuous covering layer of glomerular epithelial cytoplasm. There are four nuclei in the field of which the upper one and the lower two are external to the basement membrane and are epithelial. The fourth nucleus which lies in the center of the group is internal to the basement membrane and is endothelial. Stain, Masson's rapid method with iron hematoxylin substituted for the azo carmine in the Mallory-Heidenhain azan carmine technique.  $\times 1500$ .

FIG. 4. Two loops from a hypertensive contracted glomerulus. Case 50 (same as Fig. 3). Essential hypertension with death from rupture of the aorta in a man 47 years of age. Seventy-one per cent of his glomeruli were of the type in this illustration. The photomicrograph shows (1) a marked thickening and wrinkling of the glomerular basement membrane (contrast with Fig. 3), (2) normal glomerular epithelium external to the membrane, and (3) normal glomerular endothelium internal to the membrane. Stain, the same as in Fig. 3.  $\times 1500$ .

FIG. 5. A glomerulus showing diffuse acute inflammation. Case 21. Essential hypertension with death from cardiac decompensation in a man 55 years of age. Only 2 per cent of such glomeruli were found. The photomicrograph shows (1) an enlarged glomerulus, (2) glomerular epithelium which is swollen and undergoing hyaline granular degeneration, (3) a glomerular basement membrane of normal thinness, and (4) obstruction to the circulation by intracapillary fibers and proliferated endothelium. The loops on the right contain mononuclear cells filled with fat. (5) In the upper left portion of the field, an arteriole with an irregular subendothelial deposit of hyalin is seen. Stain, the same as in Fig. 3.  $\times 500$ .



I



2

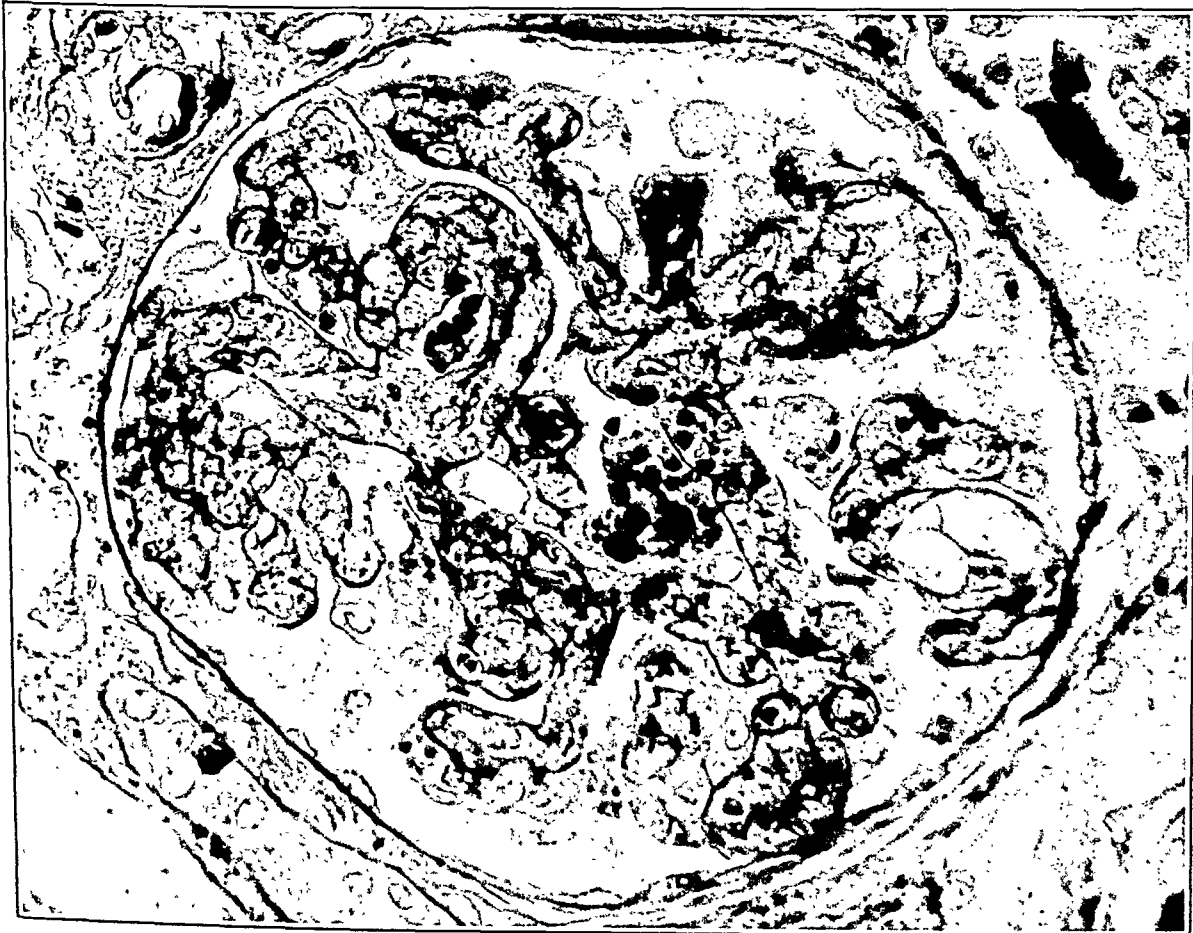




3



4



5

creased to 444,000 cells per cmm., with a differential count showing 90 to 96 per cent small lymphocytes.

It is not necessary to review again the literature relating to tumors of the lymphatic apparatus associated with leukemias since this has been adequately covered by Flashman and Leopold, who in addition have included an excellent bibliography.

### REPORT OF CASE

*Clinical History:* R. J. W., a white male, aged 61 years, steeplejack by occupation, entered the medical wards of the Boston City Hospital on November 23, 1925, complaining of distention of the abdomen, swelling of the right leg and the left ankle, and enlargement of the inguinal group of lymph nodes.

*Present Illness:* The patient was in moderately good health until about eight weeks prior to entry, when he first noticed swelling of his abdomen. About three weeks later his right leg became red and began to swell, and a week or two after this he noticed a similar swelling of his left ankle. He was indefinite about the duration of the enlarged nodes in the groin, but realized they had been present for several weeks.

*Past History:* He had recurrent attacks of bleeding hemorrhoids for eighteen years previous to an operation April 12, 1923. At this time the surgical records indicate that he had a chain of small discrete non-tender lymph nodes above Poupart's ligament on both sides. His past history was otherwise entirely negative.

*Physical Examination:* There was an enlargement of all superficial lymph nodes; this was particularly noticeable in the inguinal regions. The abdomen was distended but there was neither shifting dullness nor a fluid wave. An indefinite mass was felt in the left upper quadrant that was thought to be the spleen. The right leg and thigh were edematous and reddened; the left leg was edematous to the knee.

The rest of the physical examination including an X-ray of the chest revealed no further positive findings.

*Laboratory Examination:* The white blood cell count was 11,600 with 44 per cent lymphocytes; the red blood cells, 4,728,000, and the hemoglobin 75 per cent.

On November 27, 1925, four days after admission, the first biopsy tissue from a node removed from the left axilla was diagnosed as a "slowly growing lymphoblastoma."

### REPORT ON FIRST BIOPSY SPECIMEN

The specimen (S25-2670) consists of an encapsulated lymph node about 2 cm. in diameter. The fresh surface is homogeneous, dull, white and gelatinous.

Microscopically, in one or two areas the normal markings are entirely obliterated by a homogeneous diffuse growth of lymphocytes. Elsewhere the lymphocytes are growing in rather densely

## A CASE OF LYMPHOBLASTOMA, HODGKIN'S DISEASE AND TUBERCULOSIS \*

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In recent years the study of tumors of the lymphatic system has received unusually widespread attention both in regard to etiology and classification. Many types have been described, usually however in an uncomplicated form, but occasionally associated with tuberculosis or tumors of another kind. A case of aleukemic lymphatic leukemia that later developed lymphatic leukemia, and at autopsy showed in addition Hodgkin's disease and tuberculosis, forms the basis for this report.

Richter<sup>1</sup> recently reported a case of generalized reticulum cell sarcoma of the lymph nodes associated with lymphatic leukemia. The patient, a male 46 years of age, entered the hospital with enlarged cervical lymph nodes which had been present about seven weeks. His red blood cell count was 3,700,000, and he had a leucocytosis of 98,400, 90 per cent of which were lymphocytes. His condition became progressively worse and he died about three weeks after admission. At the time of death the reticulum cell sarcoma was the more actively growing lesion, but the wide distribution and relative quiescence of the leukemic foci indicated the long and probably earlier existence of the latter.

Still more recently Flashman and Leopold<sup>2</sup> reported a case in an elderly male beginning with a primary retroperitoneal lymphosarcoma and terminating with leukemia. Tissue obtained by biopsy from the inguinal lymph nodes that had been enlarged for twelve months revealed a lymphosarcoma. The patient was given a long series of Roentgen-ray treatments lasting over four months. The blood picture remained unchanged till the last week or two of these treatments when the white count rose to 96,000 cells per cmm., with 90 per cent small lymphocytes. The patient lived three weeks longer, and during that time the white blood count rapidly in-

\* Received for publication September 1, 1929.

103° F. On May 5, the node was incised and drained, and in forty-eight hours the temperature returned to normal.

At this time, the blood picture changed, and a count taken on May 13 showed only 10,000 white blood cells, and of these only 22 per cent were lymphocytes.

He remained in the hospital almost three months, and on June 6, 1927 when he was discharged he felt much better. The swelling of the legs had disappeared but there was still a moderate degree of ascites.

Three months more elapsed until he was readmitted on September 9, 1927, for the third time. After leaving the hospital in June he spent most of his time camping in the woods and was fairly free from symptoms till August when his ankles began to swell, and the edema gradually extended toward the body. His abdomen became more distended, the genitals were swollen and he once again developed shortness of breath.

A physical examination on his third admission again revealed considerable edema of both legs and scrotum, and marked distention of the abdomen. The blood picture at this time showed 11,000 white blood cells, with 34 per cent lymphocytes, 59 per cent polymorphonuclear leucocytes and 7 per cent monocytes.

On September 16, 1927, that is, one week after admission, his abdomen was tapped and 15,000 cc. of chylous fluid were withdrawn. An examination of this fluid showed 160,000 lymphocytes per cmm., and this high cell count was considered an adequate cause for the turbidity of the fluid.

Roentgen-ray therapy was started almost immediately, but the patient's condition grew worse, he became dyspneic and developed râles at both bases.

On November 11, 1927, an axillary lymph node was removed for biopsy and the microscopic diagnosis "suggested chronic inflammation rather than tumor but a positive diagnosis was not possible."

### REPORT OF THIRD BIOPSY SPECIMEN

The specimen (S27-2637) is a small, moderately firm, encapsulated lymph node about 2 cm. in diameter.

Microscopically this lymph node resembles the second biopsy specimen. The normal architecture of the node in places is well preserved, and there are many normal lymph nodules with well differentiated germinal centers. In addition there are other nodules composed of solid masses of uniformly mature lymphocytes. The capsule, pulp and trabeculae are infiltrated with lymphocytes, but the peripheral sinus is quite patent.

*Progress Note (Continued):* On November 18, 1927, he was again tapped and 5000 cc. of chylous fluid were withdrawn. About a week later another series of X-ray exposures was begun, each of which was followed by twenty-four hours of vomiting and diarrhoea. On December 16, 1927, a fourth lymph node was removed for examination and this was diagnosed "lymphoblastoma."

packed nodules and cords without differentiated germinal centers. There is fusion of both nodules and cords, infiltration of both pulp and capsule and almost complete obliteration of the sinuses.

The cells resemble small mature lymphocytes. There are a few lymphoblasts and a few polymorphonuclear leucocytes, including an occasional eosinophile.

*Progress Note:* The patient's condition was unchanged for about ten days and on December 5, 1925, a second lymph node was obtained for microscopic examination and diagnosed "hyperplasia of lymphoid tissue."

### REPORT ON SECOND BIOPSY SPECIMEN

The specimen (S25-2753) consists of a small, soft, red, irregular piece of tissue 2 by 1 by 1 cm.

This lymph node has many normal-appearing nodules, with well differentiated germinal centers composed of large young lymphocytes, and lymphoblasts in which mitoses are numerous. As in the previous biopsy both stroma and capsule are infiltrated with small mature lymphocytes, but the sinuses are not obliterated, and here and there one is distended with mature lymphocytes.

*Progress Note (Continued):* During the succeeding four weeks the patient received four X-ray treatments. The edema and redness of the legs disappeared but the abdomen remained distended. The white blood count varied from 14,100 on December 12, 1925, to 5,600 on December 22, 1925, with an average throughout of 50 per cent lymphocytes. While in the hospital his weight fell from 158 to 144 pounds. On January 6, 1926 he was discharged feeling better and relatively free from symptoms.

On March 25, 1927 he reentered the hospital for the second time, after being out just a little more than a year, but in the interval he was kept under observation in the out-patient department. He was fairly well until two weeks before this second admission when his abdomen became more distended, and the swelling of his legs, which had returned somewhat soon after he left the hospital, became more marked at about this time; he also developed shortness of breath on exertion.

A physical examination showed marked distention of the abdomen, shifting dullness and a fluid wave. There was general lymph node enlargement as before, but the nodes in the inguinal region were larger. His spleen was readily palpable.

The white blood count showed 59,000 cells per cmm., 92 per cent of which were lymphocytes.

The abdomen was tapped and 4000 cc. of chylous fluid were withdrawn, and for several days this fluid continued to drain away.

On April 30, 1927, an infection developed in a lymph node in his left groin; this progressed for several days causing a fever which went as high as 102° and



elapsed after this before the patient died and never during this time did the normal total and differential count show an appreciable variation.

*Clinical Diagnosis:* Aleukemic leukemia with reversal of white blood cell count.

### AUTOPSY REPORT

An autopsy (A28-319) was performed on September 8, four hours postmortem. The body is poorly developed and emaciated. The abdomen is distended and fluctuant and the lower extremities are considerably edematous. Both axillary and inguinal lymph nodes are palpable bilaterally. There is very little subcutaneous fat and the pectoral and abdominal muscles are atrophic.

*Peritoneal Cavity:* The surfaces are dull, rather opaque, and roughened by fine sand-like granulations. A thin fibrinous exudate covers the capsule of both spleen and liver. The cavity contains 4000 cc. of milk-like fluid. The mesenteric and iliac lymph nodes are greatly enlarged. The spleen is larger than normal, roughened and bound to the diaphragm by old fibrous adhesions.

*Pleural Cavities:* The left contains about 25 cc. of milk-like fluid; the right contains a similar amount, but this is watery and blood-tinged. The lower lobe of the right lung is firmly bound to the chest wall and diaphragm.

*Heart:* Weight 250 gm., is rather small and unusually soft. There is considerable gelatinous atrophic fat beneath the epicardium. The myocardium is smooth, homogeneous and dark brown. The endocardium and valves are negative, but the coronary arteries are moderately atheromatous.

*Lungs:* There are several nodules in the upper lobe of the left lung that are firm, discrete, and vary from 0.5 to 2 cm. in diameter.

*Spleen:* Weight 350 gm. It is rough, nodular and firm. The fresh surfaces are homogeneous and red showing no visible malpighian bodies.

*Gastro-Intestinal Tract:* Lying at the duodenojejunal junction is a single, firm area of chronic ulceration 2 cm. in diameter extending into and beneath the mucosa.

*Pancreas:* This is very deeply embedded in enlarged lymph nodes and connective tissue.

*Liver:* Weight 1500 gm. The capsule is loosely covered with a

## REPORT ON FOURTH BIOPSY SPECIMEN

The specimen (S27-2970) is a soft irregular piece of tissue 2 by 1 by 1 cm.

Microscopically, the normal architecture is obliterated by a diffuse growth of mature lymphocytes. The capsule is thinned and infiltrated with lymphocytes. There are no distinct lymph nodules, and only in a few places can the peripheral sinus still be made out. There is an occasional large tumor giant cell of the so-called Sternberg or Dorothy Reed type.

*Progress Note (Continued):* Soon after the last series of Roentgen-ray treatments was started he began steadily to improve.

In the latter part of January 1928 the ascites had almost cleared up and he was up and about the ward most of the time. Five or six months passed with little or no change before the lymph nodes in the inguinal region began to enlarge, and his abdomen again began to fill, but repeated blood counts taken throughout this time showed a normal number of white blood cells and normal differential values.

From about the first of August until the patient's death in September, the general condition grew progressively worse. The abdomen continued to fill and the resulting ascites had to be relieved on several occasions. His appetite diminished, he lost considerable weight and on September 8, 1928, just about three years after his first admission, he died.

## SUMMARY OF CLINICAL HISTORY

A male, 61 years of age, entered the hospital with some abdominal distention, palpable superficial lymph nodes, a normal total white blood count, but with a relatively high lymphocyte value of 44 per cent. A biopsy specimen taken at this time was diagnosed lymphoblastoma of the type consistent with aleukemic leukemia.

After an interval of about a year and a half the patient was readmitted, again showing abdominal distention and general lymph node enlargement. At this time, the white blood count was about 60,000 cells per cmm., with 90 per cent lymphocytes, and the case was diagnosed "lymphatic leukemia." He had been in the hospital only about six weeks when an acute lymphnoditis developed in one of his inguinal lymph nodes. He became acutely ill and his temperature rose to 103° F. The node was drained and after forty-eight hours he felt better and his temperature returned to normal.

At this time his white blood count was only 10,000, and his differential count taken repeatedly was normal. More than a year

*Lungs:* Sections taken through the nodules that in gross were considered to be tumor metastases, show old and recent tuberculous lesions. Irregular areas of necrosis are surrounded by a chronic inflammatory reaction in which are many solitary tubercles. The walls of a few of the medium-sized vessels are involved in this tuberculous process, and the lumina are almost completely thrombosed. The surrounding lung tissue is moderately atelectatic with the alveoli partially filled with fibrin and endothelial leucocytes.

*Spleen:* The spleen shows several different lesions. Miliary tubercles are scattered throughout the pulp. There are small discrete areas composed entirely of cells resembling those of the Hodgkin's type of tumor cell; most of these areas lie in the pulp, but occasionally they are found bulging up beneath the endothelium of the larger vessels. There are also numerous, rather discrete foci of mature lymphocytes lying beneath the endothelium of similar vessels. The connective tissue of the pulp is generally increased throughout. The endothelial cells lining the sinuses are hypertrophied and show occasional mitoses, while endothelial leucocytes within the sinuses contain both red blood cells and blood pigment. A dense layer of hyaline connective tissue covers the capsule and superimposed on this is a layer of granulation tissue containing many young tubercles.

*Liver:* Sections show numerous small, discrete and confluent foci of mature lymphocytes. Many of these foci are in and about portal areas while others are scattered throughout the lobules. Nowhere is there a suggestion of either compression or invasion of liver parenchyma.

Solitary and conglomerate tubercles are scattered throughout, and many of these are situated in the portal areas lying either along the margin or entirely within the nodules of lymphocytes.

There are also larger discrete areas composed of cells of the Hodgkin's type, similar to those already described in the spleen. These cells are growing rapidly and compressing and invading the surrounding parenchyma.

Owing to the presence of the masses of lymphocytes the bile ducts in the portal areas are elongated. Where much of the lymphoid tissue has disappeared, the increase in connective tissue and the elongation and apparent increase in the number of bile ducts produce a picture simulating that of healed obstructive cirrhosis.

delicate, lacy, fibrinous exudate. The fresh surface of the liver is reddish brown and stippled throughout with very minute fine white nodules.

*Kidneys:* Weight 200 gm. They are small and firm and the capsules strip from very finely granular surfaces. The cut surface is pale and the finer markings are poorly differentiated. Both pelves and ureters are slightly dilated; the latter pass directly through enlarged groups of iliac lymph nodes but are not grossly constricted in these areas.

*Aorta:* Superficial atheromatous ulcers are distributed throughout both thoracic and abdominal portions. The lower end of the aorta and both common iliac arteries are completely encircled by enlarged lymph nodes.

*Bone Marrow:* Both vertebral and femoral marrows are red.

*Lymph Nodes:* There is a general enlargement of the lymph nodes throughout the body, which is especially noticeable in the abdomen and pelvis. The nodes are firm, discrete, gray or grayish pink, and vary considerably in size. Some nodes show multiple minute foci of necrosis, whereas others, equally large and nearby, show minute hemorrhages but no necrosis. The largest group of nodes is in the mesentery of the small intestine; here they are adherent but quite discrete, and vary from 2 to 6 cm. in diameter. The lymphatics lying in the mesentery between the intestine and the enlarged nodes stand out as thin yellowish white lines.

*Examination of Fluid in Peritoneum:* The milky appearance completely clears on the addition of ether, and shaking. Smears of sediment stained both vitally and in fixed preparations show very many lymphocytes, some polymorphonuclear and endothelial leucocytes, and few desquamated mesothelial cells.

*Anatomical Diagnoses:* Malignant tumor of lymph nodes with metastases to liver and lung; chylous ascites; healed pleuritis; splenomegaly with chronic perisplenitis; chronic ulcer of jejunum.

#### MICROSCOPIC REPORT

*Heart:* The epicardium is composed largely of atrophic fat which contains several small foci of mature lymphocytes. The vessels are sclerosed and the muscle fibers show atrophy and considerable pigmentation.

*Lymph Node 2:* This node, which lay adjacent to the one just described, shows almost no lymphoid tissue. About the periphery is a zone of granulation tissue with a few tubercles and many tuberculous giant cells. The remainder of the node is composed simply of necrotic cellular débris.

The diagnosis of this node is tuberculosis with extensive necrosis.

*Lymph Node 3:* In this node, the normal architecture is largely replaced by a fairly homogeneous growth of mature lymphocytes. The capsule and pulp are infiltrated with lymphocytes, and the peripheral and medullary sinuses are almost obliterated. In addition, this node shows not only solitary tubercles but also an area composed entirely of large pleomorphic cells growing rapidly and invading the surrounding lymphoid tissue.

The microscopic diagnosis is probable lymphoblastoma associated with both Hodgkin's disease and tuberculosis.

*Lymph Node 4:* This node differs from the others so far described. Except for a few small islands of mature lymphocytes, it is composed entirely of cells that are rather uniform both in size and shape. They are smaller than the Hodgkin's cells and larger than lymphocytes. The cytoplasm is homogeneous and lightly basophilic. The nuclei vary somewhat in size, and the nuclear membrane is crinkled and quite irregular. There is little chromatin and this is arranged in a delicate network throughout the nucleus. The nucleolus is strikingly prominent. Mitoses in these cells are numerous and very rarely there are multiple mitoses and large multinucleated tumor giant cells.

The type cell so closely resembles the large lymphoblast that such a node may also be classified as a lymphoblastoma, but the picture is quite different from that in which the tumor cell is the small mature lymphocyte.

*Lymph Node 5:* In this node the picture is still more complicated. There are no normal landmarks except a few scattered nodules of mature lymphocytes. The remainder of the node is composed of rapidly growing tumor cells which in some fields resemble Hodgkin's cells and in others lymphoblasts, but in many places the cells are all intermingled and here it is impossible to distinguish one cell from another.

*Lymph Node 6:* This is composed almost entirely of Hodgkin's tumor cells. Both nucleus and cytoplasm of these cells vary widely

The endothelial cells lining the sinuses are hypertrophied and unusually prominent.

*Pancreas:* Solitary tubercles are distributed over the peritoneal surface and within the stroma of the pancreas. There is slight sclerosis of both larger vessels and acinar tissue.

*Parietal Peritoneum:* This is thickened by a layer of granulation tissue containing many solitary tubercles.

*Intestine:* Section from the jejunum shows a chronic ulcer extending through the submucosa into the circular muscle. Miliary tubercles are scattered along the surface of the ulcer and also along the thickened serosa.

*Kidney:* There is moderate sclerosis of the cortical portion of the kidney with atrophy and disappearance of many of the tubules.

*Adrenal:* The medulla is moderately infiltrated with mature lymphocytes and collections of endothelial leucocytes are found in the cortex.

*Aorta:* The intima shows hyaline thickening with organization of fibrin along the surface.

*Bone Marrow:* Miliary tubercles and small foci of mature lymphocytes are seen in the vertebral marrow. The femoral marrow contains a considerable amount of lymphoid tissue, in addition to showing a moderate degree of hematopoiesis.

*Lymph Nodes:* There is a wide variation in the histological picture of the many lymph nodes examined. Not only do nodes from widely separated areas differ but also those lying side by side. Furthermore, many individual nodes show not merely one, but two, and sometimes three distinct pathological lesions. Because of the varied and unusual character of the lymphoid tissue, only a few of the most typical nodes will be described in detail.

*Lymph Node 1:* This node is characterized by the presence of many nodules of mature lymphocytes. The stroma and capsule are infiltrated with these cells. The medullary and peripheral sinuses contain clusters of lymphocytes as well as many phagocytic endothelial leucocytes. There are no mitoses and no tumor giant cells.

Solitary and conglomerate tubercles are distributed in the periphery.

The histological diagnosis of such a node lies between lymphoblastoma composed of mature lymphocytes, and chronic inflammation; and in either case the picture is complicated by tuberculosis.

## DISCUSSION

This case, in some respects, is not unlike that of Richter's, and the two cases offer many problems in common. First, there is the question of whether lymphoblastoma and Hodgkin's disease should be regarded as true neoplasms; second, has the development of any one of these three lesions, namely, the lymphoblastoma composed of mature lymphocytes, that composed of large young lymphoblasts and lastly the Hodgkin's type of tumor, been dependent on the existence of either of the remaining two, or should the three types of lesions found at autopsy be considered genetically independent; third, should the aleukemic leukemia be considered the primary disease; and lastly, what relation does tuberculosis bear to the other lesions?

Histologically, the tumor composed of mature lymphocytes, which apparently involved the lymphoid tissue throughout the body, was probably the first to develop. It has a very general distribution not only in lymph nodes but also in the spleen, liver and bone marrow.

At the time of the autopsy there was almost complete cessation in the proliferation of mature lymphocytes. In lymph nodes, spleen and liver, mitoses in these cells were practically absent, and in many areas, especially in the liver, there was already considerable hyaline change. Thus, if this is to be considered a true tumor it is rapidly showing a spontaneous regression.

In regard to the large tumor lymphoblasts and Hodgkin's cells, they were multiplying rapidly and invading the surrounding tissue; each produced metastases in both spleen and liver, and in many widely separated lymph nodes.

It would appear that the patient had had for some time a slowly growing chronic type of aleukemic leukemia, and that this, perhaps as the result of an additional stimulation, exerted a demand on a more primitive cell, namely the lymphoblast, and that this in turn was followed by proliferation of a still more primitive cell, the Hodgkin's cell, which, at the time of death, was showing all the signs of a very rapidly growing malignant tumor. However, such a progression of changes may only be conjectured since the possibility of complete independence can not be disproved.

The condition of an aleukemic leukemia changing to a manifest

in size, shape and staining. The nucleolus may be single or multiple and in either case it is large, deeply basophilic and very prominent. Multinucleated tumor giant cells as well as single and multiple mitoses are common. In addition, solitary tubercles are distributed among the tumor cells.

*Lymph Node 7:* The greater portion of this node resembles a slowly growing lymphoblastoma composed of mature lymphocytes. Invading the sinuses at one end are clusters of rather uniform cells suggesting young lymphoblasts. In another field there is a mass of Hodgkin's cells extending into the surrounding lymphocytes. Lastly, solitary and conglomerate tubercles are found in among both the Hodgkin's cells and the lymphocytes. Hence, the picture is one of lymphoblastoma, Hodgkin's disease and tuberculosis.

*Microscopic Diagnoses:* Lymphoblastoma; Hodgkin's disease; tuberculosis; atrophy of the myocardium; arteriosclerosis; chronic vascular nephritis.

*Bacteriological Diagnoses:* Fluid from the peritoneal cavity was injected into four guinea pigs. Two of these were autopsied at the end of six weeks and showed generalized tuberculosis. The remaining two died at the end of eight and ten weeks respectively showing still more widespread tuberculous lesions.

Material from one of the first guinea pigs was transferred to a second and this again was transferred further, while attempts were being made to obtain cultures of the tubercle bacillus. Finally, a culture was obtained from the fifth transfer pig. The organisms grew fairly rapidly and culturally resembled either the human or bovine type of *B. tuberculosis*. A heavy emulsion of this culture was injected intravenously in a rabbit and intraperitoneally into a guinea pig. The guinea pig died seventy-four days after inoculation and at autopsy showed extensive tuberculosis of the spleen, liver, omentum and lungs. The rabbit remained well and was killed four and one-half months after the inoculation. At autopsy, the only pathological changes found were a few small tubercles at the periphery of both lungs. Microscopically, these were tubercles of the cellular type and contained a few acid-fast bacilli.

On the basis of its virulence for guinea pigs and relative avirulence for rabbits, the organism was considered a human type of tubercle bacillus.



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## DESCRIPTION OF PLATES

## PLATE 77

- FIG. 1. Liver. On the left is a tubercle, and on the right a periportal leukemic infiltration. The cells of the latter are small, adult lymphocytes.  $\times 250$ .
- FIG. 2. Lymph node. On the left is a tubercle with a Langhan's giant cell in the center. On the right is an area of Hodgkin's disease. This area consists of large cells with irregularly shaped nuclei with prominent chromatin, and of small lymphocytes. Between the two types of lesions is a band of small lymphocytes.  $\times 250$ .

leukemia is well recognized and is not an extremely unusual cytological finding; however, a case that shows these changes and then later develops a complete reversal of count is very much more uncommon. The presence of an acute infection has been reported as a cause for the reversal of count, and in this case, an acute lymphadenitis associated with a very severe systemic reaction might readily account for the change in the blood picture.

For years the frequent association of Hodgkin's disease with tuberculosis has been the subject of numerous papers, and Sternberg<sup>3</sup> as early as 1898 maintained that Hodgkin's disease and tuberculosis were caused by a common etiological agent. This problem has been carried on recently by L'Esperance,<sup>4</sup> who undertook to determine whether a relationship might exist between avian tuberculosis and Hodgkin's disease, and her report, though based on a relatively small series of experiments, at least suggests that the avian tubercle bacilli may be a factor in producing some of the lesions interpreted as Hodgkin's disease.

The human type of tubercle bacillus was isolated from this case, and it had probably been active in the body for some time since the areas of tuberculosis in the lungs were definitely chronic lesions of months or years duration. The tuberculous lesions in the liver, spleen and lymph nodes on the other hand resembled miliary tubercles and were of recent origin. It is possible that the tubercle bacillus may have borne some relation to the initiation of the original tumor of the lymphatic apparatus, and even more probable that it modified the subsequent cytological and histological course. However, these are simply hypotheses which still remain to be proved.

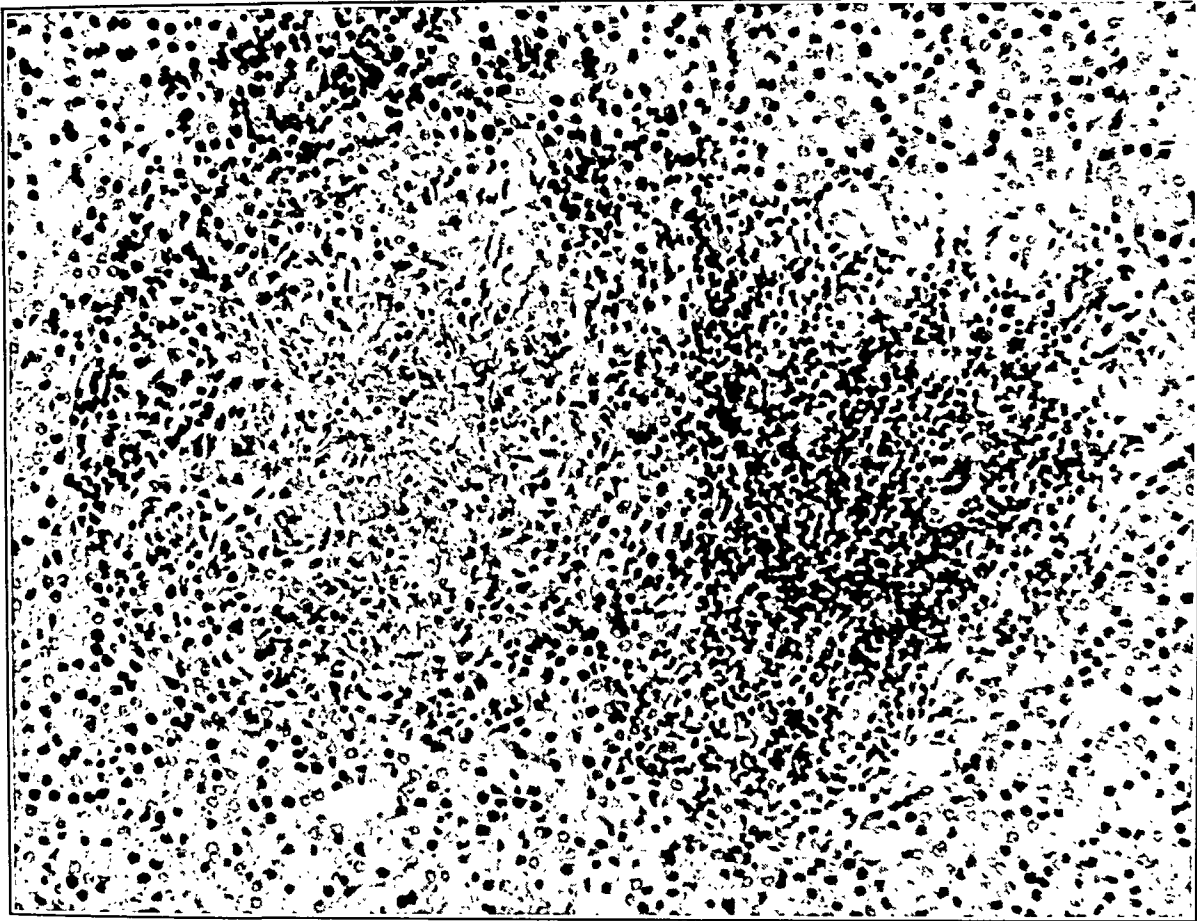
#### SUMMARY

A case is described of an elderly patient who was admitted to the hospital with the diagnosis of aleukemic leukemia. He was watched over a period of three years, and during that time he developed a manifest lymphatic leukemia. Following a short but severe infection, his white blood count and differential values returned to normal and remained unchanged till his death a year and a half later. He came to autopsy, and examination showed that he had not only aleukemic leukemia, but also Hodgkin's disease and generalized tuberculosis.

PLATE 78

FIG. 3. Liver. On the right is a nodule of Hodgkin's disease. On the left is a leukemic infiltration. The latter is being invaded by the cells of the former.  $\times 250$ .

FIG. 4. Lymph node. An area of Hodgkin's disease. In the upper left corner is a tumor giant cell; below it is a multiple mitosis. At the right is another multiple mitosis. The majority of the cells shown are of the same type with irregular nuclei and prominent nucleoli. There are also some small lymphocytes scattered among these Hodgkin's tumor cells.  $\times 500$ .

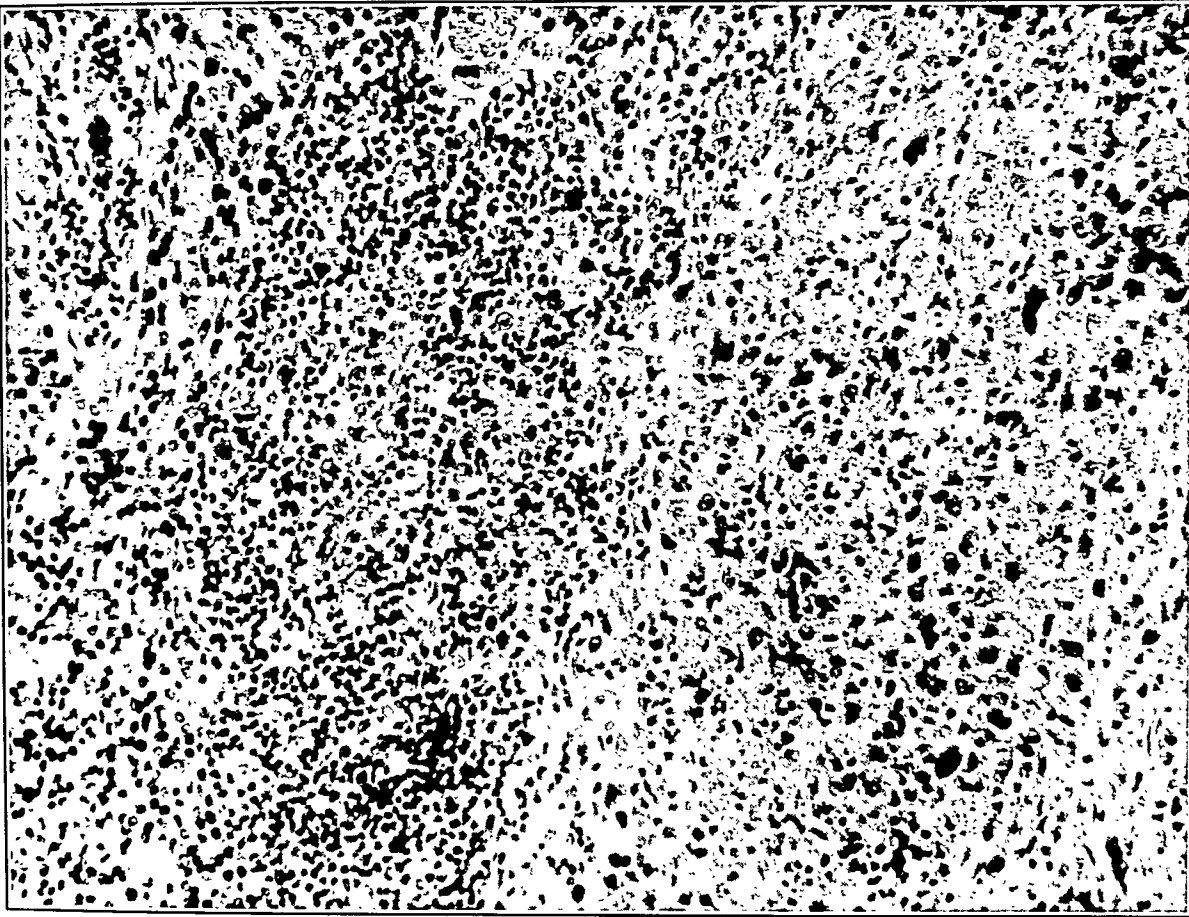


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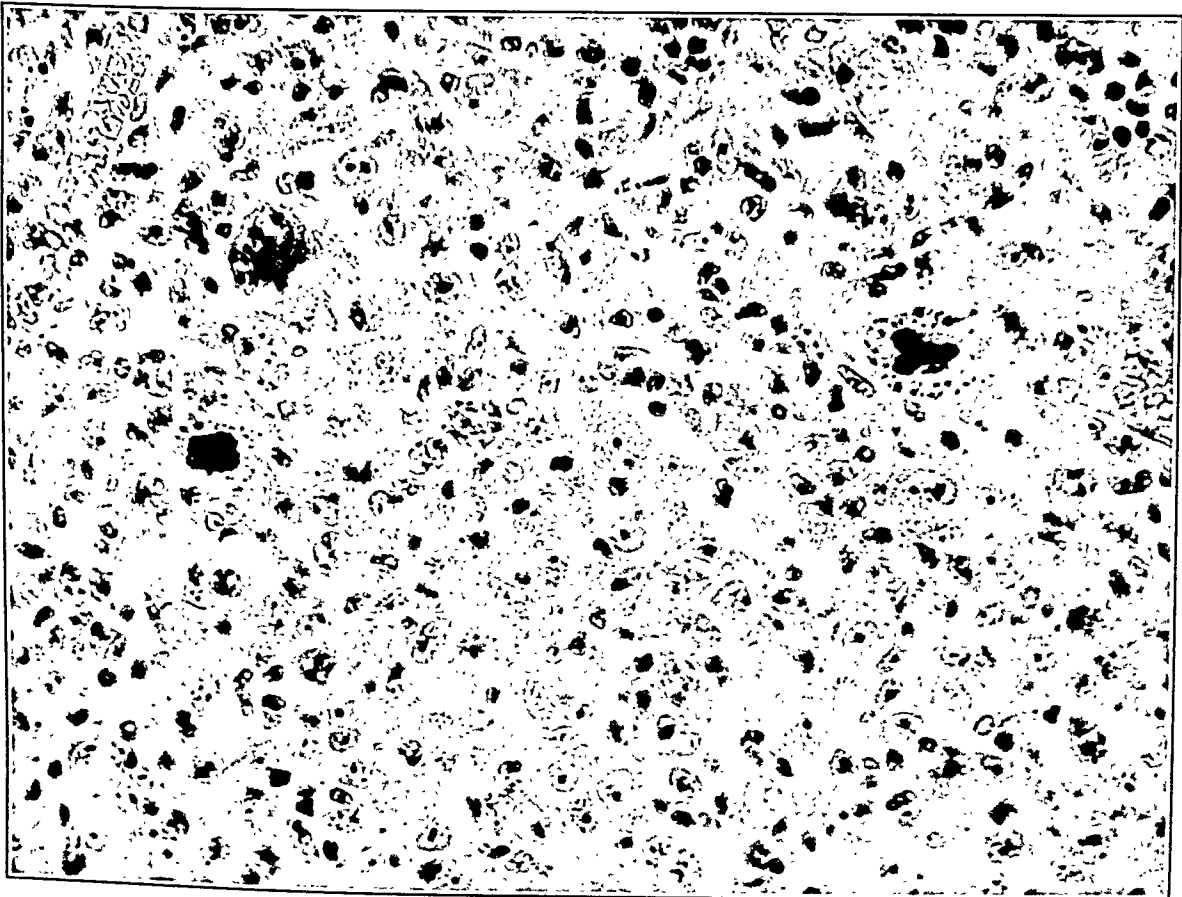


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cultures 5 cc. For subsequent injections the former was increased to 5 cc., then to 10 cc., and the latter to 10 cc. With the control injections of Berkefeld filtrates of broth cultures and with the injection of heat-killed filtrates of other organisms, the dosage and time intervals were the same as with the dead streptococci, except with *B. coli* and *B. tuberculosis* where the initial dose was 1 cc. The tubercle bacilli were grown for three weeks on coagulated egg medium and a heavy saline suspension was used. Cultures were usually killed by heating at 60° C for 20 minutes and were always tested before injection by subculturing to suitable media. As a rule, a minimum of 6 doses was given. The guinea pigs were usually injected through one of the superficial leg veins; the initial dose was 0.5 cc. and subsequent doses increased to a maximum of 5 cc., although frequently only 2 cc. could be injected due to the difficulty of injection. Supravital stains and total leucocyte counts of the circulating blood were made on many of the animals before and after the injections.

All animals, whenever possible, were killed painlessly with ether and autopsied immediately. Fresh smears from the cut surfaces of the organs were frequently taken and stained by the supravital technique. The tissues were fixed in Zenker's solution and in 10 per cent formalin. Routine sections were made from the Zenker-fixed tissue in paraffin and stained with eosin and methylene blue. When advisable, sections were stained by the Gram Weigert method and by Foot and Mènard's<sup>3</sup> modification of Hortege's silver carbonate method.

The majority of animals that received injections of killed bacteria showed no ill effects; there were no pronounced losses in body weight or of appetite. A few of the animals died during the course of injection; in some no apparent cause could be found, others probably died from the effects of endotoxins, such as those of *B. coli*, and in others the characteristic lesions in the lungs were present to such a degree as to produce pulmonary circulatory changes of sufficient gravity to be considered a contributory cause of death. Total white blood corpuscle counts and supravitaly stained (neutral red) differential counts of the circulating blood were made before and after injections on many of the animals. The majority of these rabbits were those that were to receive or had received injections of killed non-hemolytic streptococci. As a rule, there was a moderate rise in total count with a moderate to marked increase in the absolute number of monocytes and a corresponding decrease in the number of lymphocytes. Some rabbits showed no appreciable changes. The maximum percentage of monocytes was 39 per cent (Rabbit No. 25, 24 hours after the ninth injection), and many of the bloods gave figures higher than 20 per cent, as compared with a maximum of 10 or 12 per cent before injection. The results on two groups of rabbits, other than those receiving the non-hemolytic streptococci, are of interest. Two rabbits, Nos. 27 and 28, which were injected

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## TISSUE REACTIONS IN RABBITS FOLLOWING INTRAVENOUS INJECTION OF BACTERIA \*

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Reference has been made in a recent paper<sup>1</sup> to the peculiar reaction in rabbit tissues to repeated injections of non-hemolytic (gamma type) streptococci. A preliminary report<sup>2</sup> of the general distribution and histology of these lesions has been made. In general, the lesions are characterized by the mobilization, in various organs of the body, of large mononuclear cells which appear to be monocytes, according to supravital classification, and which quite frequently lead to the formation of giant cells of the Langhans' type. Because of the unusual and peculiar lesions, because of the type of cell called forth and because of the nature of the antigen which results in this response, the observation would appear to be of interest to the pathologist, cytologist and immunologist.

The present paper deals with the methods used to produce the lesions and with a detailed description of the histological changes. Different methods of injection have been tried, various organisms have been tested for their ability to produce the changes, and other animals, namely, guinea pigs, have been utilized.

### EXPERIMENTAL

#### *Intravenous Injection*

*Method:* Injections were made into the ear veins of rabbits (average weight 1800 gm.) at intervals of four or five days. With living 24 or 48 hour broth cultures of the gamma type streptococci the initial dose was 1 cc., and with dead

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three or four cells of the lymphoid type, beneath the endothelium of the larger arteries. The changes which occur in the capillaries of the alveolar walls are marked, but difficult to follow. There is a thickening of the alveolar walls, and in various areas the alveoli are obliterated. Fairly large foci of lymphoid cells are found and smaller foci of the large mononuclear cells and giant cells. The latter appear to originate from lesions in or about the smaller venules and capillaries, similar to those found in the veins. Large phagocytic mononuclear cells, some of which are similar in staining reaction to those in the veins and alveolar walls, are found free in the alveoli. All the normal alveolar structure may disappear and be replaced by areas of true consolidation. Smears from the cut surface of such lungs, stained by the supravital method, show a predominance of cells classified as monocytes. The giant cells have the typical monocytic "rosette." In sections from lungs obtained within a few hours after injection and stained by the Gram Weigert method, the organisms can be easily demonstrated. They are phagocytized most often by mononuclear cells which are usually within, or in close relation to, the capillaries of the alveolar wall. Occasional polymorphonuclear cells are seen which contain the bacteria. The organisms have never been observed in the multinuclear cells which are found in the characteristic subendothelial vascular lesion or in those free in the alveoli. Many more bacteria are found in the lungs than in the liver and spleen. Animals killed one day, or longer, after the last injection show few, if any, organisms.

*Liver:* After two or three intravenous injections small islands of lymphoid cells are found in the sinusoids of the liver lobules, chiefly at the periphery. Mononuclear cells with large pale ovoid nuclei appear in these islands and eventually giant cells are formed. There is a generalized increase of cells between the liver columns, involving the cells of the sinusoidal endothelium and connective tissue cells. The columns of parenchymal cells are narrowed, particularly at the periphery of the lobule, but there is no apparent necrosis. Occasionally tiny projections into the lumina of the larger hepatic veins are observed which are similar to the very early lesions in the pulmonary veins. Supravitaly stained smears of freshly cut sections show many mononuclear and multinuclear cells which are classified as monocytes. In Gram Weigert stained paraffin sections from rabbits killed within a few hours after the last injection, many bacteria are seen phagocytized by the normal appearing endothelial (Kupffer) cells of the sinusoids; none are found in the large mononuclear or multinuclear cells.

*Spleen:* After the third intravenous injection clumps of large mononuclear cells are seen in the splenic pulp adjacent to the terminal veins or splenic sinuses. Eventually these increase in size, and frequently such collections contain numerous giant cells. Oftentimes they are found adjacent to the splenic nodules. In the advanced form they closely resemble miliary tubercles. The germinative centers of the splenic nodules are usually extremely active, showing a large percentage of pale immature cells and many mitoses. In Gram Weigert stained sections, the organisms are found in the relatively large phagocytic cells ("splenic cells") of the normal spleen. As these cells phagocytize leucocytes, one cannot say whether the bacteria are taken up first by the splenic cells or whether they have been phagocytized primarily by phagocytic leucocytes. Fewer organisms are found in the spleen than in the lung and liver from the same animal.

*Kidney:* Giant cells are sometimes found in the glomerular tufts.

with filtrates from broth cultures of non-hemolytic streptococci, showed moderate increases in monocytes (22 and 19 per cent, respectively) on the day following the fifth injection with a gradual decline to normal, subsequently. Two other rabbits, Nos. 85 and 86, showed no abnormality of the circulating blood leucocytes on the day following the sixth injection of massive doses of dead tubercle bacilli.

The sera of rabbits that had received six or more injections of living non-hemolytic streptococci usually showed definite macroscopic agglutination against the homologous organisms in 1:1280 or 1:2560 dilutions.

At autopsy the only abnormalities noted in animals that had received a sufficient number of injections of dead organisms to result in marked microscopic lesions, were failure of the lungs to collapse on opening the pleural cavities, with an increase in consistency on sectioning and moderate to marked increase in the size of the spleen.

The variations in reaction of the same species of animal to different organisms, of a different species of animal to the same organism, and so forth, are of interest. In order to be able to describe accurately such variations it will be necessary to give a detailed histological description of the characteristic lesions produced in the rabbit following intravenous injection.

*Lungs:* After the second or third injection the most characteristic changes are focal collections of cells about large and small veins. These foci consist of cells which appear to be lymphocytes and resemble those of the peribronchial lymph nodules which are normally present, except that the cells are fewer in number and appear to be less mature; mitoses are fairly common. At this time the alveolar walls show some increase in cells which are apparently normal in type. After the third or fourth injections, obvious venous lesions make their appearance. These consist of collections of cells in the loose subendothelial connective tissue of the intima, and are usually in close relation to the perivascular foci of lymphoid cells. The cells in the earliest lesions appear to be of the lymphocytic series, but, as the lesions progress the cells have the characteristic appearance of monocytes, according to ordinary staining and to the supravital classification, and giant cells of the Langhans' type are often present. The infiltration may proceed to complete occlusion of the lumen, particularly in the smaller veins, and this results in a lesion very similar to a tubercle. The larger veins are never completely occluded, but frequently show a marked infiltration at both sides of the points of entrance of smaller veins, suggesting that the cells of the exudate have pushed their way up underneath the endothelium from the smaller into the larger vessel. In all instances the lining endothelium is intact, and true thrombi are only rarely observed. Lesions of this sort are never found in the arteries or arterioles, except for an occasional tiny focus, consisting of

TABLE I

Animal		Organism	Culture	Doses	Fate of animal	Time after last injection	Tissue reactions		
No.	Kind						Lung	Liver	Spleen
1	Rabbit	Streptococcus, gamma type, "Small A"	Living	6	Killed	9 days	-	o	o
2	"	"	"	5	Died	24 hours	++++	++++	++
23	"	"	"	6	"	24 "	++++	++++	++++
24	"	"	"	9	Killed	24 "	++++	++++	++++
25	"	"	"	9	"	3 days	++++	++++	++++
26	"	"	Dead	6	Died	5 hours	++++	++++	++++
27	"	Berkefeld filtrate of "Small A" culture	"	9	Killed	4 days	o	o	o
28	"	"	"	6	Died	3 "	o	o	o
29	"	Streptococcus, gamma type, "Small A"	Dead	2	Killed	4 "	+	=	+
30	"	"	"	2	"	4 "	+	+	+
31	"	"	"	3	"	5 "	+	++	++
32	"	"	"	3	"	5 "	++++	++++	++++
33	"	"	"	4	"	4 "	++++	++	++++
34	"	"	"	4	"	4 "	++++	++++	++++
43	"	"	"	16	Died	24 hours	+, old	+, old	-
44	"	"	"	6	Killed	30 days	o	o	o
45	"	"	"	4	Died	2 "	++	=	+
48	"	"	"	6	Died	4 "	++++	++++	++
49	"	"	Dead, 100°C	12	Killed	5 "	++++, old	++++, old	++
50	"	"	Dead	6	"	2 "	++++	++++	++
53	"	"	"	8	"	24 hours	++++	++++	-
55	"	"	"	8	"	24 "	++++	++++	++

*Pancreas:* Negative.

*Adrenal:* Giant cells are rarely found in the *zona fasciculata* between the columns of parenchymal cells.

*Small Intestine:* Negative.

*Mesenteric Lymph Nodes:* Negative, except for hyperplasia.

*Heart:* In several animals subendothelial lesions similar to, but less pronounced than, those found in the lungs have been observed in one or more of the medium-sized veins draining the myocardium.

*Bone Marrow:* The bone marrow, as a rule, is hyperplastic, without any particular change in cell types. Rarely true giant cells, as distinguished from megakaryocytes, are found.

*Skeletal Muscle:* Negative.

*Brain:* Negative.

A list of the animals injected intravenously is given in Table I. As the characteristic lesions were found most commonly in the lungs, liver and spleen, the results of microscopic examination of sections of these organs have been used to determine whether or not the injections produced the changes in question.

The lesions described above were first noted<sup>1</sup> in rabbits that had been injected with living cultures of non-hemolytic (gamma type) streptococci for the purpose of obtaining agglutinating sera. One strain ("Small A") was chosen as representative of those organisms capable of producing the reaction in the rabbit and an attempt was made to obtain further information by varying certain factors. Heat-killed (60° C for 20 minutes or 100° C for 20 minutes) cultures were found to produce the same lesions as the living cultures, except, of course, for the occasional acute vegetative endocarditis found in animals injected with the latter. Provided the animal was killed within five or six days after the last injection, definite changes in the lungs and liver were always found following two intravenous injections, and marked changes occurred after five or more doses. Two rabbits, Nos. 1 and 44, that were killed nine and thirty days respectively after the last injection, and that had received a sufficient number of injections to produce, ordinarily, pronounced lesions showed no histological changes of note. Two rabbits, Nos. 43 and 49, that received 16 and 12 injections of killed organisms and were killed one and five days, respectively, after the last injection, had definite lesions in the lungs and liver, but they were much less marked than those found in animals after five or six doses, and the presence of a certain amount of fibrosis gave evidence of regression and repair. Two rabbits, Nos. 27 and 28, that received the Berkefeld

filtrate from killed cultures identical with those used to inject two rabbits, Nos. 25 and 26 in which typical reactions were found, showed no demonstrable lesions. Three rabbits, Nos. 82, 83 and 84, that, after their fifth dose of organisms, received daily doses of lithium carmine up to and past the sixth dose of bacteria, showed lesions which were less marked than those usually found at that period. The distribution of carmine, together with its significance, will be discussed in a subsequent paper.

Three guinea pigs, Nos. 80, 87 and 88, were successfully injected intravenously with killed cultures of "Small A." The only lesions of any significance were found in the lungs. The majority of small and medium-sized veins were surrounded by collections of lymphoid cells similar to those found in the early changes in rabbits. No abnormal cells were observed within the vessels, and, neither cells of the monocytic type nor giant cells could be demonstrated. Sections of the livers and spleens showed nothing remarkable. Two of the three sections of the adrenals showed large areas of necrosis resembling infarcts. One of these showed an acute inflammatory reaction, while the others showed merely necrosis.

Rabbits were injected intravenously with cultures of six other strains of non-hemolytic (gamma type) streptococci. Three of these strains ("Birk. 2," "N.T. 21," and "N.T. 16") produced typical reactions in the lungs, livers and spleens. The rabbits that received two other strains ("Small B," and "N.T. 2-8") were killed so long after the last injection, fifteen and eleven days respectively, that one would not expect to find any lesions, and none were found. The one other strain ("R.F.T. 4") failed to have any effect.

A green-producing diplococcus ("B-26-27"), isolated from the stool of a case of chronic ulcerative colitis, and a strain of *Streptococcus viridans*, isolated from the blood of a case of bacterial endocarditis, were injected without effect. The latter grew so poorly that the result is hardly comparable because of the relatively small number of organisms injected. With *Streptococcus scarlatinae* ("Dick I") definite lesions were obtained, most marked in the lungs, whereas with a strain of *Streptococcus hemolyticus* the changes in the liver and the spleen were the most prominent.

The injection of killed cultures of *B. coli* resulted in changes identical with those previously described. The lesions in the liver appeared to be more marked than those in the lungs, as compared

	Rabbit	Streptococcus, gamma type, "Small A"	Dead		Killed	3 days				
65	"	"	"	3	Died	3 days	++	±	±	±
77	"	"	"	6	"	"	+++	+++	+++	+++
78	"	"	"	8	"	"	+++	+++	+++	+++
80	Guinea pig	"	"	6	Killed	"	+	±	±	±
82	Rabbit	Streptococcus ("Small A") + carmine	"	6	Died	"	+++	+++	+++	+++
83	"	"	"	6	"	"	+++	+++	+++	+++
84	"	"	"	6	Killed	"	+++	+++	+++	+++
87	Guinea pig	Streptococcus, gamma type, "Small A"	"	6	"	"	+	±	±	±
88	"	"	"	6	"	"	+	±	±	±
5	Rabbit	Streptococcus, gamma type, "Small B"	Living	6	Killed	15 days	-	+, old	-	-
9	"	"	"	6	Died	"	+++	+++	+++	+++
12	"	"	"	5	Killed	"	o	o	o	o
22	"	"	"	12	"	"	o	o	o	o
46	"	"	Dead	6	"	"	+++	+++	+++	+++
47	"	"	"	6	Died	"	+++	+++	+++	+++
35	"	Diplococcus, green-producing, "B-26-27"	"	6	Killed	"	o	±	±	±
36	"	"	"	6	"	"	±	o	o	o
38	"	Streptococcus scarlatinae, "D I"	"	6	"	"	+++	+++	+++	+++
59	"	Streptococcus hemolyticus	"	6	"	"	+	+	+	+
60	"	"	"	6	"	"	+	+	+	+
63	"	Streptococcus viridans	"	6	"	"	±	±	±	±
64	"	"	"	7	"	30 min.	o	o	o	o
58	"	Bacillus coli	"	6	"	4 days	+++	+++	+++	+++
62	"	"	"	4	Died	24 hours	++	+++	+++	+++
85	"	Bacillus tuberculosis, bovine	"	6	Killed	2 days	+++	+++	+++	+++
86	"	"	"	6	"	"	+++	+	+	+

- = not done, o = negative, ± = questionable, + = very slight, ++ = slight, +++ = moderate and ++++ = marked.

cells and giant cells. The lesions in the lungs were much less marked than those following the injection of bacteria, but nevertheless identical changes could be demonstrated. The pigmented mononuclears and giant cells were found chiefly in relation to the capillaries in the alveolar walls and to the peribronchial and perivascular collections of lymphoid cells. Definite subendothelial lesions consisting chiefly of pigmented macrophages could be demonstrated in one or more of the medium-sized veins of each specimen, but they were relatively slight as compared with those previously described. Little or no pigment was found in the endothelial cells of the capillaries and larger blood vessels.

### *Intraperitoneal Injection*

Two rabbits, Nos. 39 and 40, were injected intraperitoneally with killed cultures of gamma type streptococci ("Small A"). The initial dose was 5 cc. and this was increased to 10 cc., then to 20 cc., with injections at four or five-day intervals. One rabbit, No. 39, developed "snuffles" and died on the day following the fifth injection; there was a marked loss of weight. The other rabbit, No. 40, which showed a moderate loss of weight, was killed on the second day following the sixth injection. The findings at autopsy were identical in both instances. All the organs in the peritoneal cavity were bound together and to various portions of the parietal peritoneum by friable fibrinous adhesions. There was a marked general thickening of the peritoneum in which were many pin-point to pin-head-sized pale yellow foci which contained a firm pale yellow exudate. The omentum was greatly thickened and contained a moderate number of these foci. The mesenteric lymph nodes were somewhat enlarged. There was no free fluid. All organs, on section, proved negative except for the thickened visceral peritoneum about the abdominal organs. Cultures from the peritoneal cavity and heart's blood yielded no growth. Smears taken from the peritoneum showed a marked increase in the number of cells with a predominance of small and large mononuclear cells.

The lungs, liver and spleen, on microscopic examination, showed none of the changes observed following intravenous injection; in fact, the only deviation from normal histology was observed in the peritoneum. In sections from the omentum a marked thickening

with the same lesions following the injection of gamma type streptococci.

Two rabbits, Nos. 85 and 86, were injected with suspensions of dead bovine tubercle bacilli. In these animals the most pronounced lesions were in the lungs, although a few giant cells were found in the spleens and a moderate number in the livers. The pulmonary lesions were somewhat different from those previously described. There were many foci of large pale staining mononuclear cells and giant cells, surrounded by zones of lymphoid cells. These areas had apparently arisen from lesions in small veins or capillaries and looked like true miliary tubercles. Subendothelial lesions in the larger veins were present, but were not as prominent as in other rabbits. In sections stained to demonstrate the tubercle bacilli, the organisms were found in the lungs in the giant cells and in the mononuclear cells, both of the tubercle-like foci of the subendothelial lesions and of those occurring singly in alveolar walls. In the livers the bacilli were found occasionally in the giant cells and in the small groups of mononuclear cells which were apparently the forerunners of the giant cells. The giant cells and the large pale mononuclear cells in the spleen contained a few acid-fast rods and granules. Intradermal tuberculin tests in both animals, before and after the series of injections, were negative.

Through the kindness of Dr. Gulli Lindh Muller, sections of organs of rabbits that had received repeated injections of colloidal substances, such as India ink and collargol, were made available for study. Furthermore, sections from rabbits that had received repeated intravenous injections of collargol and that had been previously described by one of the authors in conjunction with Stewart<sup>4</sup> were restudied. In rabbits that had received 6 to 12 injections, the lesions in the livers and spleens were practically identical with those found following the injection of killed bacteria and the distribution of pigment was the same as that of the dead tubercle bacilli. There were many pigmented giant cells in the livers, chiefly in the sinusoids at the periphery of the hepatic lobules, but also at the edges of the periportal spaces. Furthermore, the majority of endothelial cells lining the sinusoids contained pigment. In the spleens the germinal centers were hyperactive; and, amongst the lymphoid cells at the periphery of the germinal centers, in the splenic pulp and, occasionally, free in the sinusoids, were numerous pigmented mononuclear



edema. One of these lesions was removed on the seventh day and showed a walled-off abscess without any surrounding reaction. The cells in the abscess were predominantly mononuclears, but the phagocytized bacteria which still persisted were found in polymorphonuclear cells.

### *Intradermal Injection*

Several rabbits, either normal or previously injected intravenously, were injected with 0.2 cc. of a heated culture of a non-hemolytic streptococcus ("Small A") intradermally. All showed after twenty-four and forty-eight hours a tiny yellow nodule at the site of injection, without any surrounding erythema.

### DISCUSSION

When the lesions, which have been described, were first observed in tissues from rabbits that had received injections of living non-hemolytic (gamma type) streptococci, it was thought that they represented a specific reaction to infection with the organism used. Subsequent work, however, showed clearly that identical changes resulted following the injection of dead organisms of various sorts and, to a less degree, particularly in the lung, to injections of particulate matter, such as India ink and collargol. It would appear that these lesions represent the non-specific reaction of the rabbit in the process of disposing of certain foreign materials injected into the blood stream. That the changes are temporary and result in no serious permanent damage is shown by the essentially negative findings in animals killed a week or more following the last injection. The ability of the rabbits, at least in the case of killed bacteria, to dispose of the injected material, following many injections, without requiring as marked a degree of tissue reaction as at first, is evidenced by the relatively mild lesions in animals that received as many as 12 to 16 injections. These facts would seem to indicate that the primary response is that of a normal animal, rather than that of an immunized animal, or of one with its tissue reactions altered due to repeated injections resulting in allergic manifestations. The mononuclear cell, characteristic of the lesions, is intimately associated with tissue changes such as those of tuberculosis which are usually considered to be of an allergic nature and the time intervals

composed of very active atypical granulation tissue was observed. There were many engorged young blood vessels and numerous fibroblasts undergoing mitosis. Many of the larger blood vessels were surrounded by collections of lymphoid cells, but there were no sub-endothelial lesions. Scattered throughout the young connective tissue were a few polymorphonuclear leucocytes and many lymphocytes and plasma cells. In addition there were many large mononuclear cells, some of which were undergoing mitotic division, and in certain areas there were a few giant cells of the Langhans' type. The pale yellow foci, observed grossly, consisted of areas containing many of the cells, mentioned above, packed closely together. The centers of such foci usually showed necrosis with an accompanying predominance of polymorphonuclear leucocytes.

### *Subcutaneous Injection*

Subcutaneous injection of 1 cc. of a killed culture of a non-hemolytic streptococcus ("Small A") into the anterior abdominal wall of a normal rabbit resulted, after twenty-four hours, in an area of marked brawny edema measuring 20 by 30 mm., with slight erythema. After forty-eight hours the animal was anesthetized and the lesion removed. Microscopically the lesion showed a small abscess surrounded by a diffuse acute inflammatory reaction extending from the corium down through the muscle layers, with necrosis of many fibers of the latter. When stained by the Gram Weigert method a moderate number of the polymorphonuclear leucocytes were found to contain bacteria.

After six intravenous injections the same rabbit was injected again, subcutaneously. There was practically no induration after twenty-four hours, and after forty-eight hours only a small nodule at the site of injection remained. This lesion was also removed on the second day and on examination showed a rather definitely localized abscess with only a moderate amount of surrounding acute inflammatory reaction. The cells in the abscess were predominantly polymorphonuclear leucocytes, but there were many mononuclear cells. The former contained many phagocytized bacteria, many more apparently than those in the previous lesion.

Two other rabbits injected subcutaneously, subsequent to intravenous injections, showed local swellings without surrounding

or locally, of certain foreign materials not necessarily toxic, rather than to infection. The rise of circulating monocytes following the intravenous injection of non-hemolytic (gamma type) streptococci is undoubtedly a reaction of similar nature.

The reactions in the liver and spleen which have been described in this paper, are practically identical histologically with those seen following the intravenous injection of lamp black, India ink, collargol, colloidal iron, and so forth. Such lesions have been accurately pictured in papers by McJunkin,<sup>21</sup> Foot,<sup>22</sup> Polson<sup>23</sup> and Stewart and Parker.<sup>4</sup> Following the intravenous injection of minute daily doses of silica sol in rabbits, Gye and Purdy<sup>24</sup> have described marked endothelial changes in the liver and spleen and some in the glomeruli, lungs, cortices of the adrenals, and reticulum of the lymph nodes, which consisted of compact masses of endothelial cells with occasional giant cells and which were ascribed to the toxic action of the silicon ion. The pulmonary subendothelial lesions in the small veins and venules are quite inconspicuous following such injections, and have usually failed to attract attention. Foot<sup>22</sup> noted in splenectomized animals some thickening of the alveolar walls which he considered to be due to the swelling of the capillary endothelium and, to a lesser degree, to the accumulation of mesenchymoid cells in the interstitial tissue; frequently cells resembling tumor giant cells were present.

Many contributions have been made relative to the tissue changes following the intravenous injection of bacteria or bacterial products. The older papers deal, chiefly, with the lesions following the intravenous injection of dead tubercle bacilli or their products (Prudden and Hodenpyl,<sup>25</sup> Vissman,<sup>26</sup> Klett,<sup>27</sup> de Giava,<sup>28</sup> Morse and Stott<sup>29</sup> and Jaffé<sup>30</sup>). In general, using dead tubercle bacilli, lesions were found in the lungs, liver and spleen which resembled true tubercles except for the absence of caseation. Identical lesions were obtained, however, with extracts of tubercle bacilli,<sup>27, 28, 29</sup> and with other organic materials,<sup>30</sup> and the consensus of opinion seemed to indicate that the active principle was some toxic substance normally contained in the tubercle bacillus. No mention is made of subendothelial venous lesions in the lungs, but Vissman<sup>26</sup> noted that the *arterial* walls were thickened and infiltrated with round cells, in places so marked that the lumina were nearly obliterated, and Morse and Stott<sup>29</sup> called attention to the fact that the endothelial cells of the

of the injections of the rabbits are such that one might expect a resulting allergic state. Intradermal and subcutaneous test injections, however, in rabbits that had received previous intravenous injections showed decreased, rather than increased, tissue reactions, and experiments such as those of Zinsser<sup>5</sup> and Swift and Derick,<sup>6</sup> have shown that allergy to bacteria or bacterial products usually does not follow intravenous injection, presumably because of the absence of focal lesions. A recent article by Ehrich,<sup>7</sup> in which identical sub-endothelial lesions are described in rabbits three to seven days following the intravenous injection of relatively large single doses of killed staphylococci, is additional proof that the reaction is that of a normal animal.

Monocytosis has been observed in experimental animals, and, in some instances, has been associated with certain infections such as tuberculosis (Sabin and Doan<sup>8</sup> and Camp, Luton, Tompkins and Cunningham<sup>9</sup>), Virus III disease in rabbits (Pearce and Casey<sup>10</sup>) and a disease in rabbits produced by *B. monocytogenes* (Murray, Webb and Swann<sup>11</sup>). Similar increases, however, have followed the injection into rabbits of acid-fast bacilli, such as, *B. smegmatis* (Jones and Tirrill<sup>12</sup>), *B. phlei* (McJunkin<sup>13</sup>) and *B. leprae* (Schwartz and Cunningham<sup>14</sup>), which are ordinarily considered non-pathogenic, the injection of phosphatide fractions of human tubercle bacilli (Sabin and Doan<sup>15</sup>) and the injection of dead cultures of *B. monocytogenes* (Witts<sup>16</sup>). Furthermore, a marked periodic increase in circulating monocytes following the injection of colloidal substances, including protective colloids, has been noted by Simpson.<sup>17</sup> Lawrence, Tompkins and Cunningham<sup>18</sup> were able to produce lesions resembling tuberculosis by the subcutaneous injection of many supposedly inert substances. Ray and Simpson<sup>19</sup> injected guinea pigs subcutaneously and into the lungs with living tubercle, grass and colon bacilli and with defatted bacilli or with lipins from the same organisms. Identical tubercle-like lesions were obtained with all these organisms or materials, with the exception that caseation was observed only with living tubercle bacilli. Very recently Wright<sup>20</sup> has described the local production of monocytes and Langhans' giant cells following the subcutaneous injection of various non-irritating gases, such as oxygen, nitrogen and carbon dioxide. A consideration of these observations would lead one to believe that such increases in monocytes are due to the presence in the blood stream,

mal, as a whole, to some foreign material. That the reaction is not one of sensitization or of immunization is further substantiated by the observation that apparently identical lesions are produced by a substance, silica sol, which contains no nitrogen. The question arises as to whether such changes represent proliferative reactions, secondary to phagocytosis, on the part of the various cells of the body which belong to the reticulo-endothelial system, whether they are produced as a result of the toxic action of the substances injected or products formed in the body from such substance, or whether they arise from a combination of the two.

In general, the sinusoidal endothelium of the liver and spleen is considered to be the most important tissue for removing foreign substances from the blood stream. The capillary endothelium of the lungs has practically no phagocytic ability. Several investigators, however, have reported findings which indicate that the tissues of the lungs, at least, in certain animals, do exhibit marked phagocytic properties. Wyssokowitsch,<sup>37</sup> following the intravenous injection of bacteria, obtained numerous organisms from the lung at a time when blood cultures were negative, and Werigo<sup>38</sup> reported the same finding. Drinker and Shaw<sup>39</sup> injected finely dispersed manganese dioxide intravenously into cats and after one hour were able to recover from the lungs 47 per cent of the material injected. Polson<sup>23</sup> recovered large amounts of iron from the lungs of rabbits following intravenous injection of colloidal iron, but attributed the result to the presence in the blood vessels of emboli containing large amounts of the colloid. Hopkins and Parker<sup>40</sup> injected relatively large doses of living streptococci intravenously in cats and rabbits and were usually able to recover more organisms from the lungs than from any of the other organs. Mole<sup>41</sup> studied the reticulo-endothelial system of the rabbit by observing the phagocytosis of stained foreign erythrocytes and called attention to the importance of the lung in this system of the rabbit. Recent work in this laboratory has shown that, following the intravenous injection of large doses of bacteria, there is a marked increase of polymorphonuclear leucocytes in the lungs. Such an increase in cells, many of which, under these conditions, usually contain large numbers of phagocytized bacteria, might account for the recovery from the lungs of so many organisms. The facts, however, that collargol, India ink and other colloidal suspensions are found in the lungs in mononuclear and giant cells

capillaries in the lungs proliferated to such an extent that there was obliteration of the lumina and that the normal endothelial tubes were changed to syncytial cords. The more recent papers are concerned, chiefly, with a comparison of the tissue reactions in normal and sensitized animals. Following the intravenous injection of hen's red blood corpuscles in guinea pigs, Oeller<sup>31</sup> observed an increase in the peri-arterial collections of lymphoid cells in the lungs and spleen, desquamation of the capillary endothelium of the lungs and spleen to form monocytes, and marked endothelial changes in the arteries and veins. He interpreted these lesions as toxic changes secondary to the phagocytosis and destruction of the foreign corpuscles and noted that they appeared sooner in animals that had received repeated injections. Following repeated intravenous injections of dead or living *B. coli* in rabbits, Siegmund<sup>32</sup> noted endothelial changes in the liver, spleen, lungs and marrow. These consisted, mainly, in proliferative changes and in the formation of intravascular thrombi composed of large pale cells with vesicular nuclei, and subintimal and subendothelial reactions to these thrombi were often present. Domagk<sup>33</sup> injected specific shocking doses into mice rendered anaphylactic to live or dead staphylococci, streptococci, *B. coli* or to serum, and reported marked endothelial changes in the liver and lungs. In the liver, cell knots were formed in the sinusoids resulting in compression of the parenchyma, and, in the lung, the proliferative changes resulted in compression of the capillaries. The latter changes were considered to be sufficient to interfere mechanically with respiration and to produce death. After single and repeated injections of killed staphylococci injected intravenously in rabbits, Ehrich<sup>7</sup> has described marked mesenchymal reactions which in distribution and in composition are identical with those described in this paper and which he attributed to the primary reaction of normal rabbits to the bacteria or their products. Seemann,<sup>34</sup> Gerlach and Finkeldey<sup>35</sup> and Gerlach and Haase,<sup>36</sup> however, using essentially the same methods, have failed to observe, particularly in the lungs, any marked histological changes which they consider as pathognomonic.

It is apparent that tissue changes closely resembling those described in this paper have been observed in experimental animals following the intravenous injection of any of a variety of substances, and that such changes represent a non-specific reaction of the ani-

The tissues of guinea pigs injected intravenously with non-hemolytic (gamma type) streptococci in doses comparable to those given rabbits failed to show any of the characteristic lesions in the lungs, liver and spleen. There was apparently an increase in the perivascular collections of lymphoid cells in the lungs, but monocytes and giant cells were never found in any of the organs. This would seem to suggest that the method of disposal in the guinea pig is different from that of the rabbit or that the bacteria in the doses given failed to stimulate any proliferative monocytic activity.

In conclusion one may say that the rabbit reacts in a very definite way to the intravenous injection of colloidal substances and of large amounts of certain kinds of bacteria. This foreign material is removed from the circulation chiefly in the sinusoids of the liver and spleen and in, or about, the capillary network of the lungs. This process of phagocytosis stimulates the production of cells which have the staining reactions of lymphoid cells and which apparently are eventually converted into monocytes and giant cells, and result in lesions which closely simulate those of tuberculosis. Such changes are most pronounced in the lungs, liver and spleen, but giant cells of the Langhans' type can be found in the bone marrow, glomeruli of the kidney and cortex of the adrenal. If the injected material is relatively stable (India ink, collargol and *B. tuberculosis*), it is found in the monocytes and giant cells, but one cannot say whether this phagocytosis is primary or whether cells already containing phagocytized material are, in turn, taken up. Such organ changes are usually accompanied by an increase of the monocytes in the circulating blood. The type of material injected seems to determine the organ in which the lesions are most prominent. With colloidal substances the lesions in the liver and spleen are most marked, with gamma type streptococci those in the lungs and liver, with *B. coli* those in the liver and with *B. tuberculosis* those in the lungs. The lesions produced by bacteria usually disappear promptly when the injections are stopped or when the rabbits become "immune" due to repeated injections, leaving little or no evidence of damage, other than a mild degree of fibrosis. The changes in the lungs following the injection of *B. tuberculosis* are more severe in type, probably due to the liberation of toxic substances from the slowly disintegrating bacilli, and, if such animals had been observed a longer time after the last injection, some definite damage to the lung structure would undoubtedly have occurred.

and that in these experiments the bacteria were observed chiefly in the same types of cells, would seem to indicate that the mononuclear cells, rather than mobilized polymorphonuclear leucocytes, are responsible for the phagocytic activity of the lungs, at least in the rabbit. In the past many writers have claimed that the capillary endothelium in the lungs possessed great phagocytic powers, but few, at present, hold to this view. In the experiments described in this paper phagocytized bacteria were never observed in the lining endothelium of the blood vessels.

The presence of the mononuclear and giant cells in the various organs raises the question as to their origin. There is no evidence that the monocytes in the lungs are derived from the circulation or from the capillary endothelium. If they arose from cells normally present in the intima, such as connective tissue cells or histiocytes, one would expect to find numerous mitoses in the lesions, which was not the case. It is certain that the subendothelial lesions in the lung are preceded by perivascular collections of cells which have the staining properties of lymphocytes and that groups of similar cells constitute the initial lesions in the sinusoids of the liver. Furthermore, the earliest subendothelial pulmonary lesions are composed of cells of this type. If it is true that the monocytes are derived from these cells, this fact would confirm the contention that monocytes may originate from lymphoid cells, an idea brought forth by Maximow<sup>42</sup> and, more recently, supported by Bloom.<sup>43</sup> This would imply that the cells invade the relatively thin adventitial coats of the veins or migrate along the venules between the endothelium and muscular coats, starting at points in the capillary bed where the latter are negligible. The first possibility was never substantiated by the observation of actual invasion of the adventitia by perivascular cells, whereas the peculiar bulging up of the endothelium in large veins at the point of entrance of smaller ones certainly suggests the second. From a study of the lungs of rabbits injected with India ink or collargol it would appear that these substances are removed from the circulation and phagocytized by cells intimately connected with the capillary network and that the pigments are transported by these cells to different portions of the pulmonary interstitial tissue. Lucid explanations of the origin of the monocytes and the mechanism of formation of the subendothelial lesions must, for the present, be foregone.



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## DESCRIPTION OF PLATES

### PLATE 79

- FIG. 1. Lung (No. 31). Perivascular collection of lymphoid cells. Very early subendothelial lesion. Eosin-methylene blue.  $\times 500$ .
- FIG. 2. Lung (No. 32). Two early subendothelial lesions, one composed chiefly of lymphoid cells and the other of monocytes. Eosin-methylene blue.  $\times 250$ .

## SUMMARY

1. Following the intravenous injection into rabbits of relatively large doses of various dead bacteria, there is a marked reaction of the tissues which contain cells of the reticulo-endothelial system. This reaction consists in an increase of lymphoid cells which are eventually transformed into, or replaced by, monocytes and giant cells. Such lesions, ordinarily, are temporary and result in no permanent damage.

2. Identical lesions occur after the intravenous injection into rabbits of various colloidal substances.

3. Such changes represent the reaction of normal rabbits in the disposition of foreign materials in the blood stream and have nothing to do with reactions secondary to sensitization or immunization.

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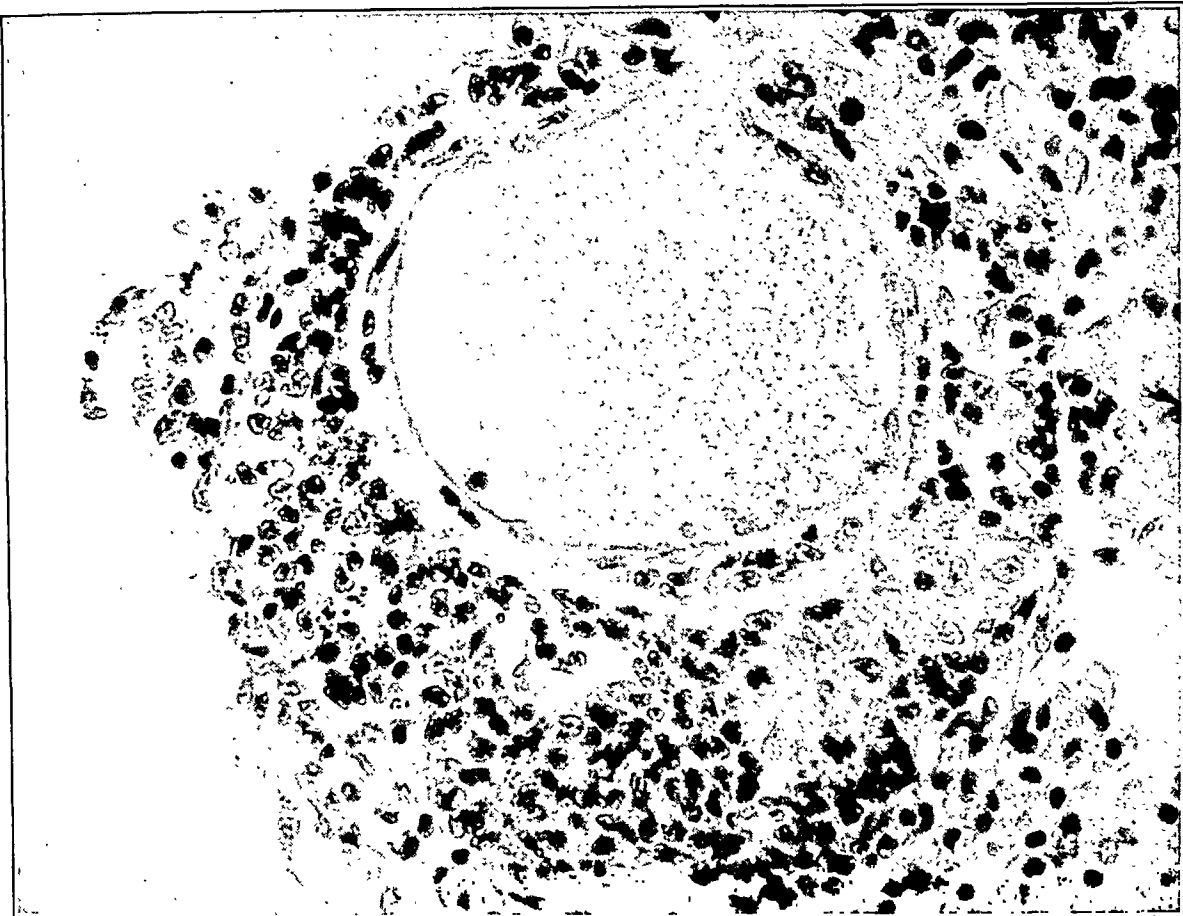
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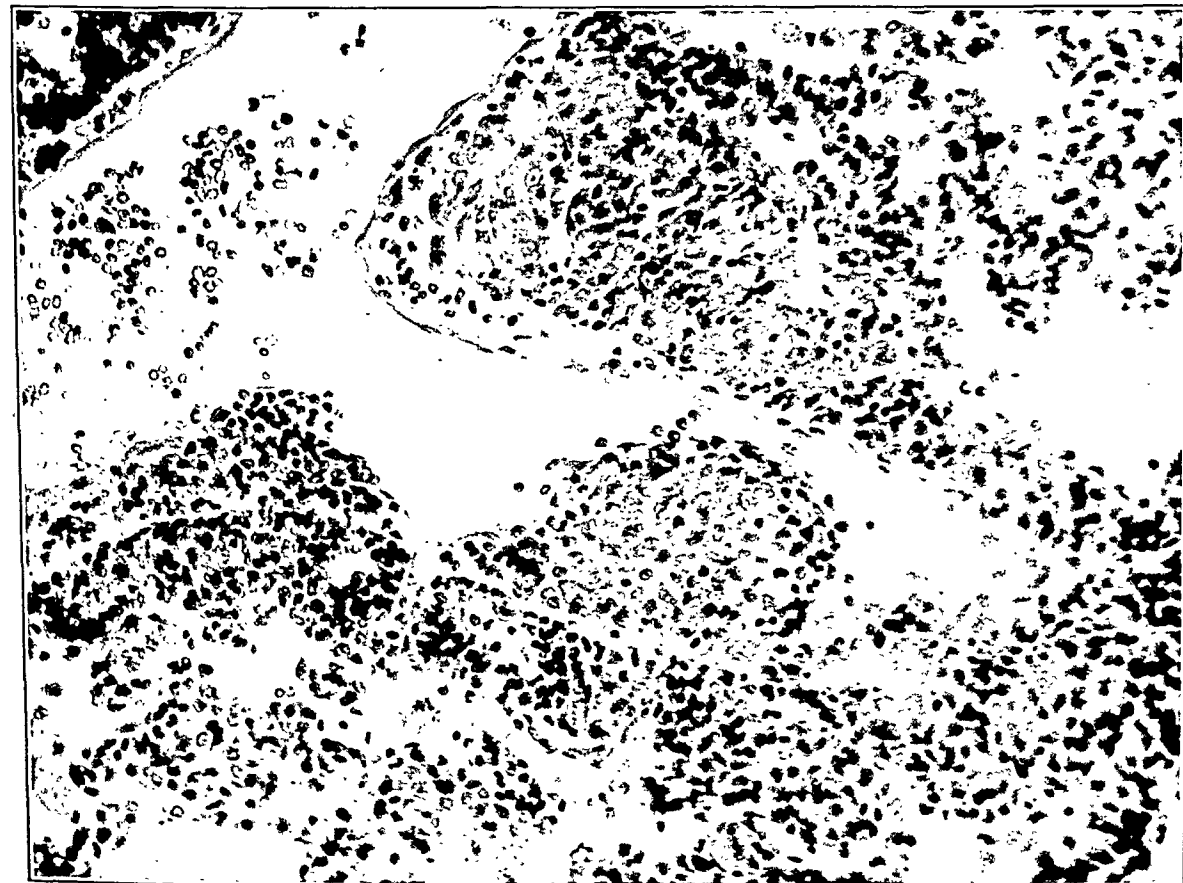
PLATE 80

FIG. 3. Lung (No. 34). Subendothelial lesion, composed chiefly of monocytes. Eosin-methylene blue.  $\times 500$ .

FIG. 4. Lung (No. 34). Subendothelial lesion with many monocytes, a few lymphoid cells and occasional giant cells. Eosin-methylene blue.  $\times 200$ .



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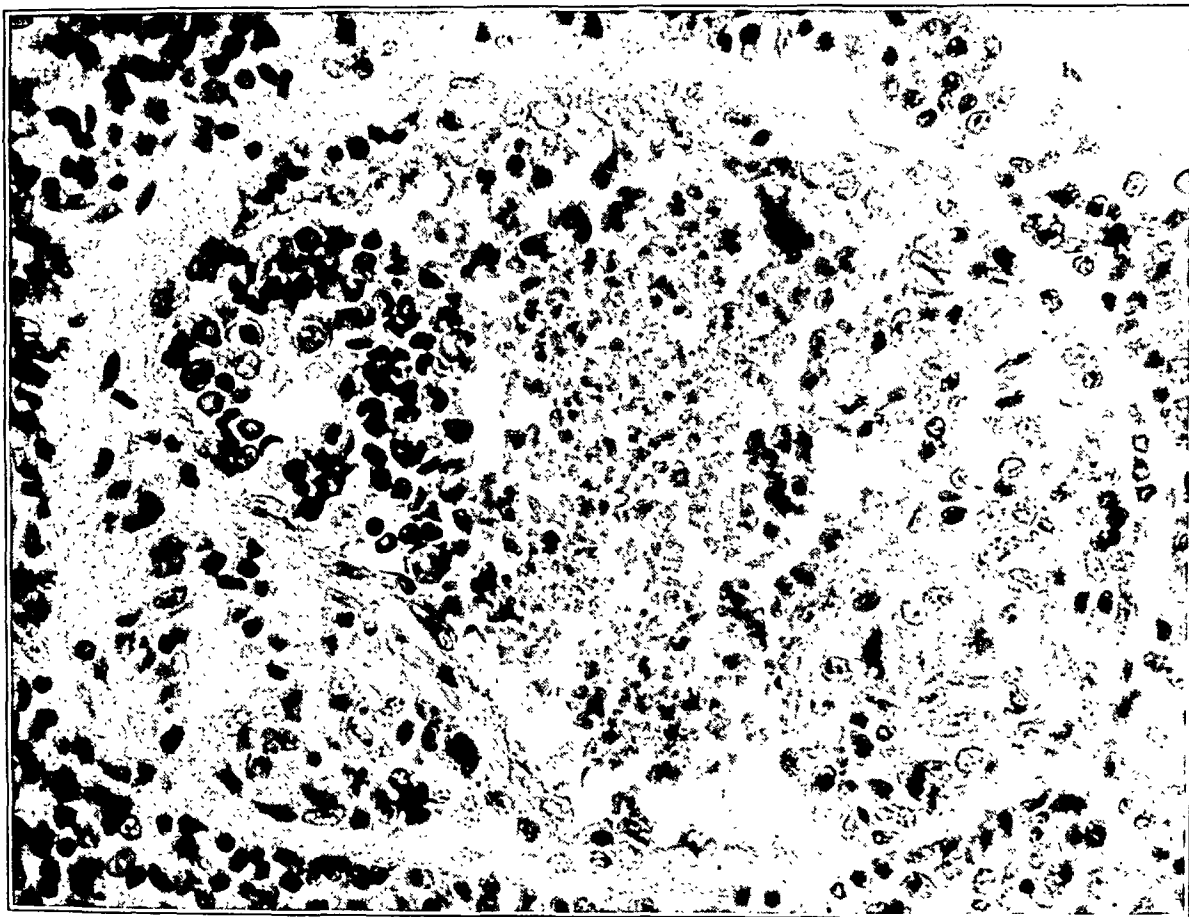


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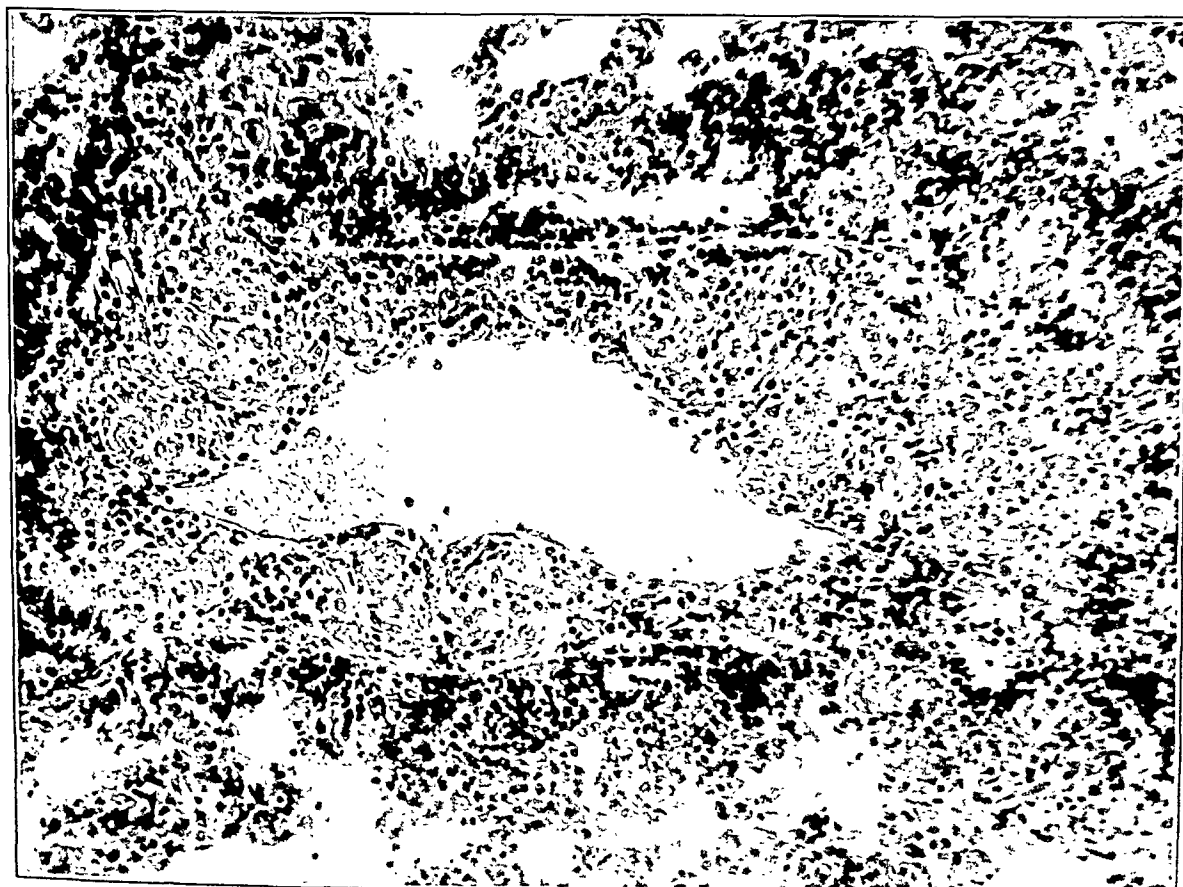
PLATE 81

FIG. 5. Lung (No. 46). Almost complete obliteration of the lumen of the vein. Lesion consists chiefly of monocytes and giant cells. Eosin-methylene blue.  $\times 500$ .

FIG. 6. Lung (No. 34). Subendothelial lesion with vessel wall emphasized by the staining of reticulum. Foot and Mènard's modification of Hortega's silver carbonate method.  $\times 500$ .



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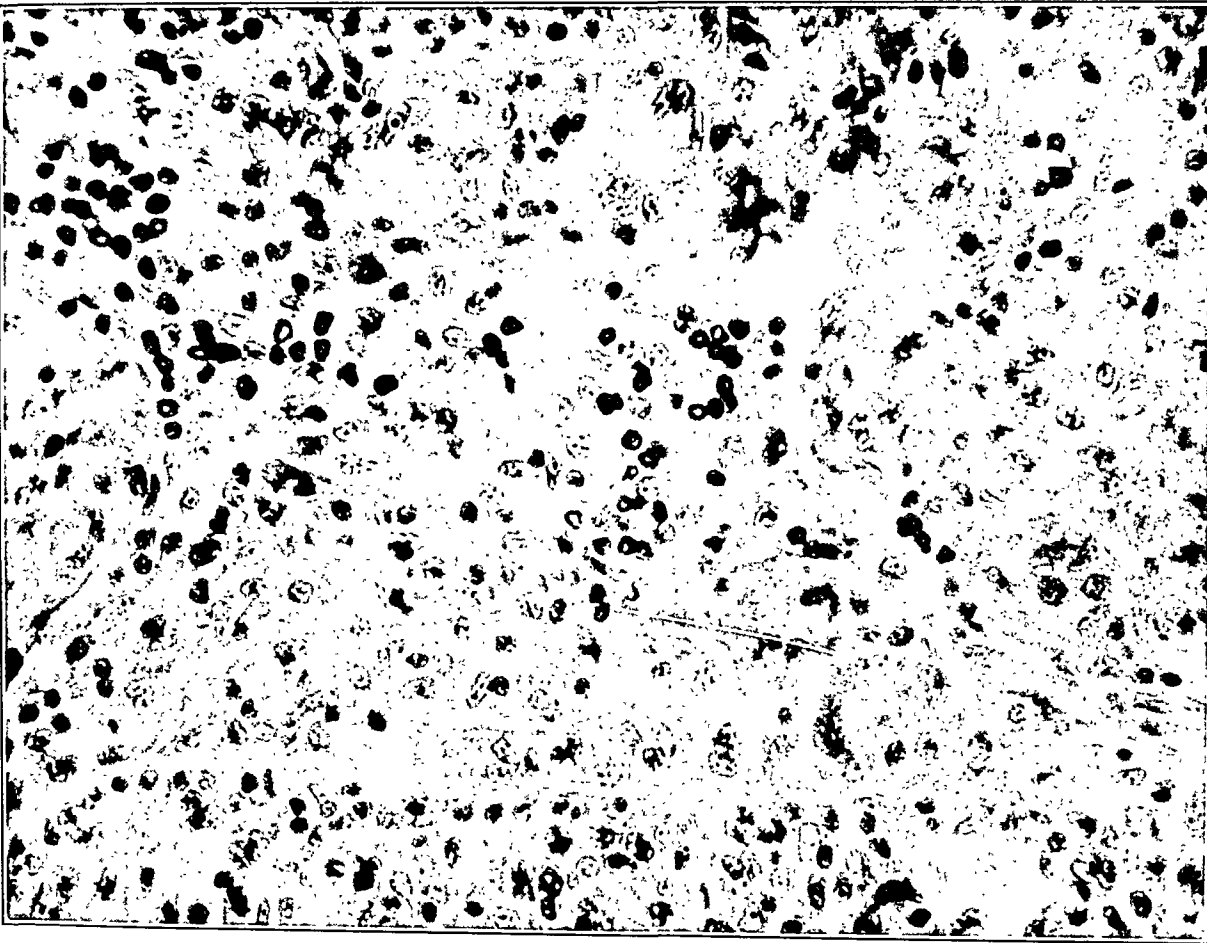


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PLATE 82

FIG. 7. Lung (No. 58). Subendothelial lesion. Eosin-methylene blue.  $\times 300$ .

FIG. 8. Lung (No. 86). Early subendothelial lesion, chiefly lymphoid cells with a few monocytes. Eosin-methylene blue.  $\times 500$ .



5



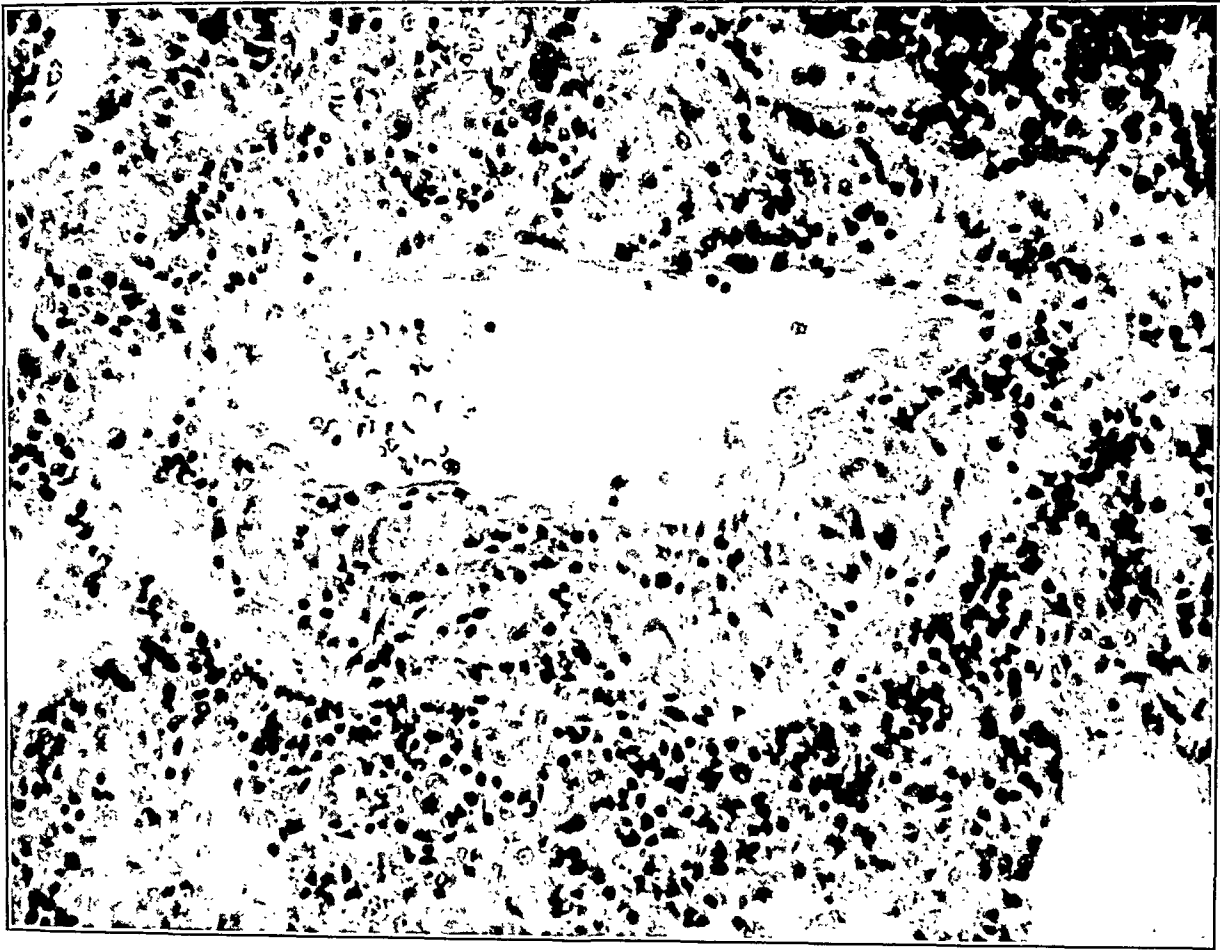
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PLATE 83

FIG. 9. Lung (No. 86). Nearly complete occlusion of the vein by monocytes with a marked perivascular accumulation of lymphoid cells. Eosin-methylene blue.  $\times 500$ .

FIG. 10. Lung (No. 86). Complete occlusion of the vessel by a lesion simulating a true tubercle. Eosin-methylene blue.  $\times 500$ .



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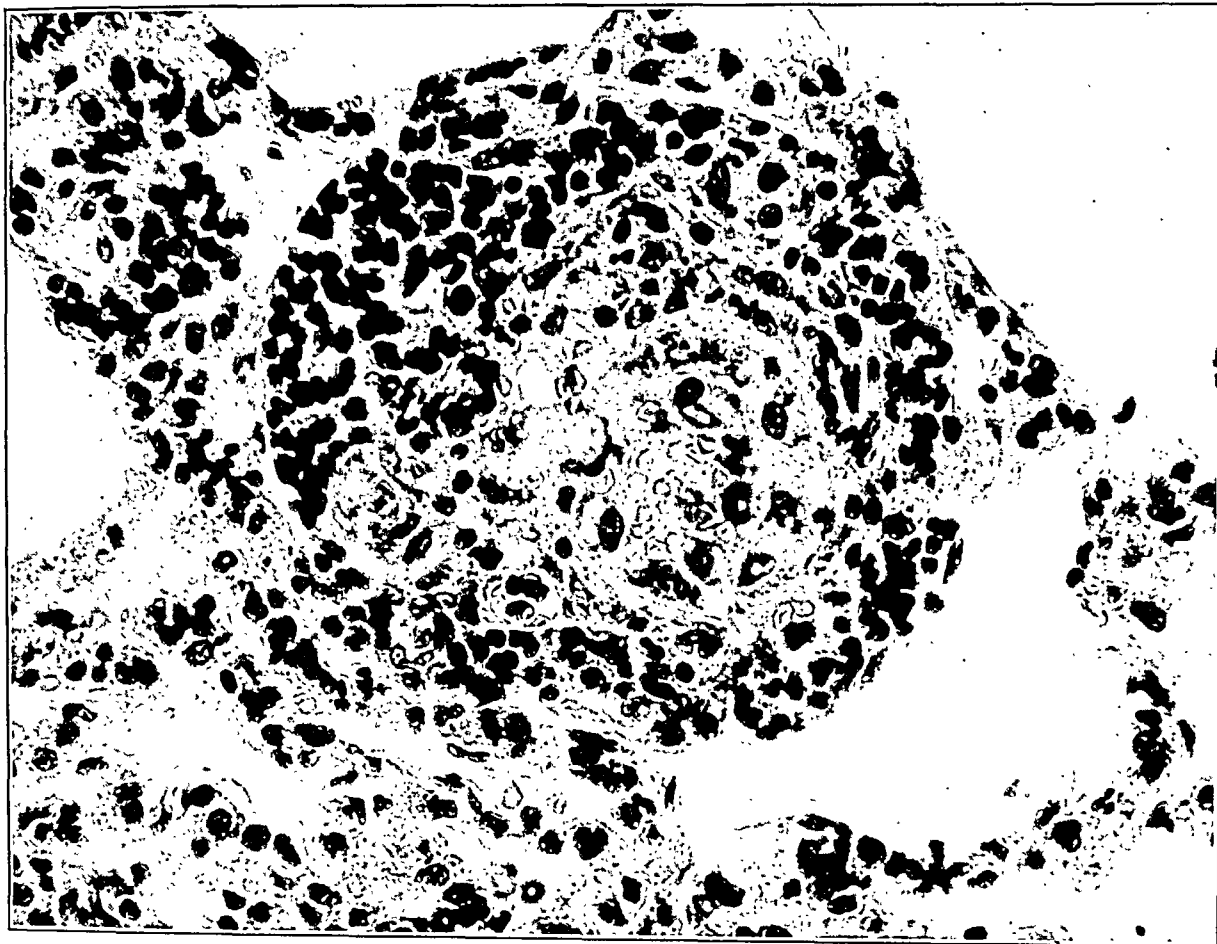


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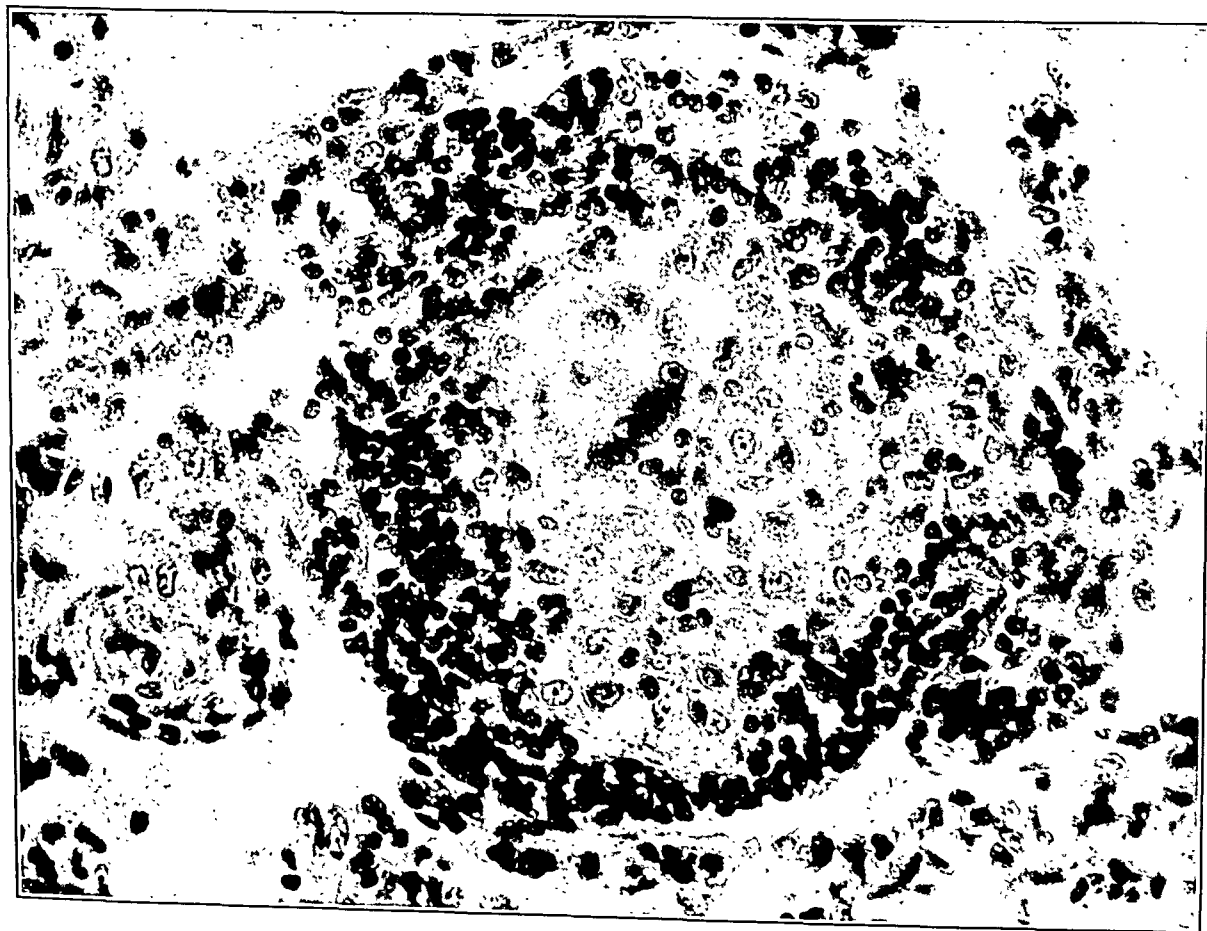
PLATE 84

FIG. 11. Lung (12 intravenous injections of India ink at weekly intervals). Marked perivascular collection of lymphoid and pigmented cells, also subendothelial lesion composed chiefly of latter. Eosin-methylene blue.  $\times 250$ .

FIG. 12. Lung (No. 38). Complete obliteration of the smaller veins with an apparent subendothelial extension of the process up into the large vein. Eosin-methylene blue.  $\times 100$ .



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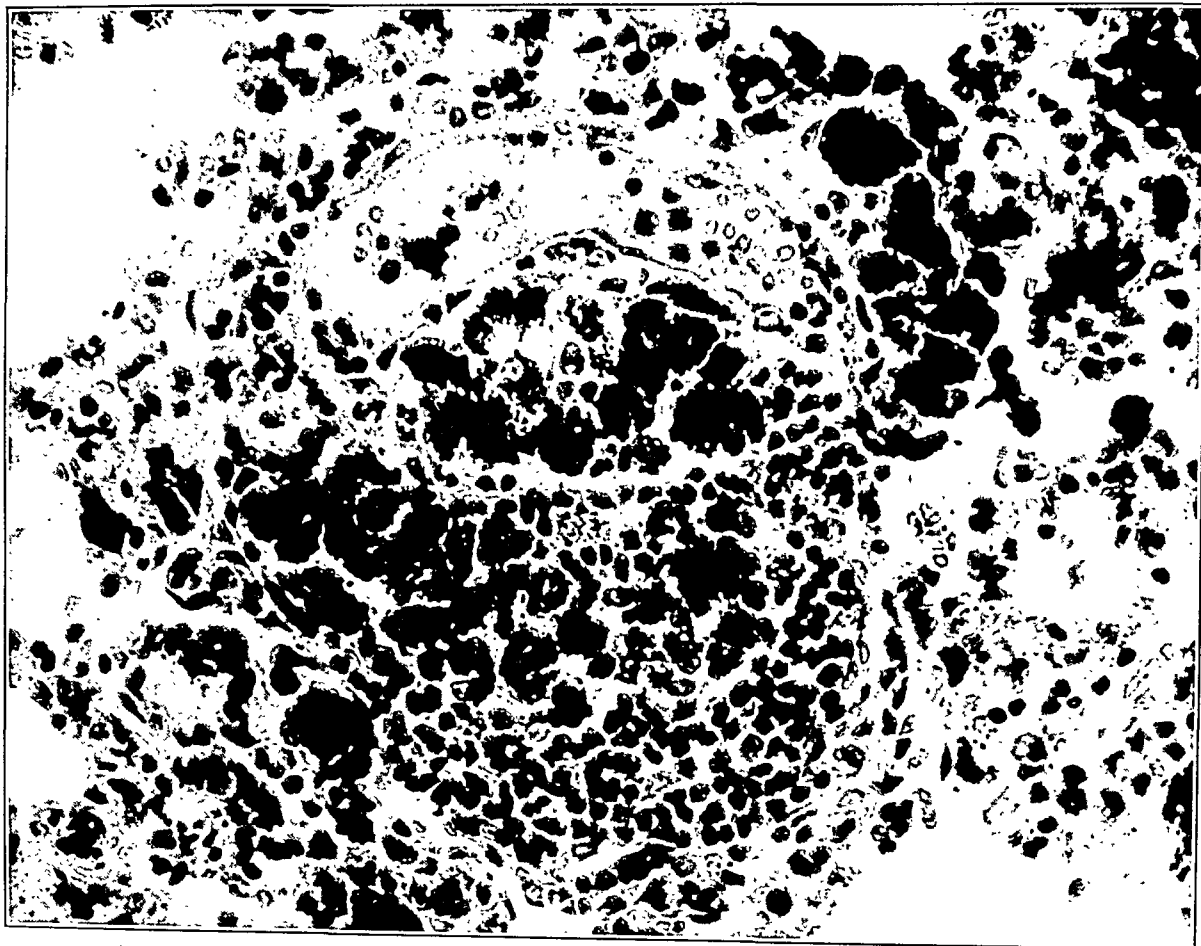


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PLATE 85

FIG. 13. Liver (No. 23). Small groups of lymphoid cells in the sinusoids, together with monocytes and giant cells. Eosin-methylene blue.  $\times 250$ .

FIG. 14. Liver (No. 23). Monocytic "rosette" in a giant cell. Foot and Mènard's modification of Hortega's silver carbonate method.  $\times 2000$ .



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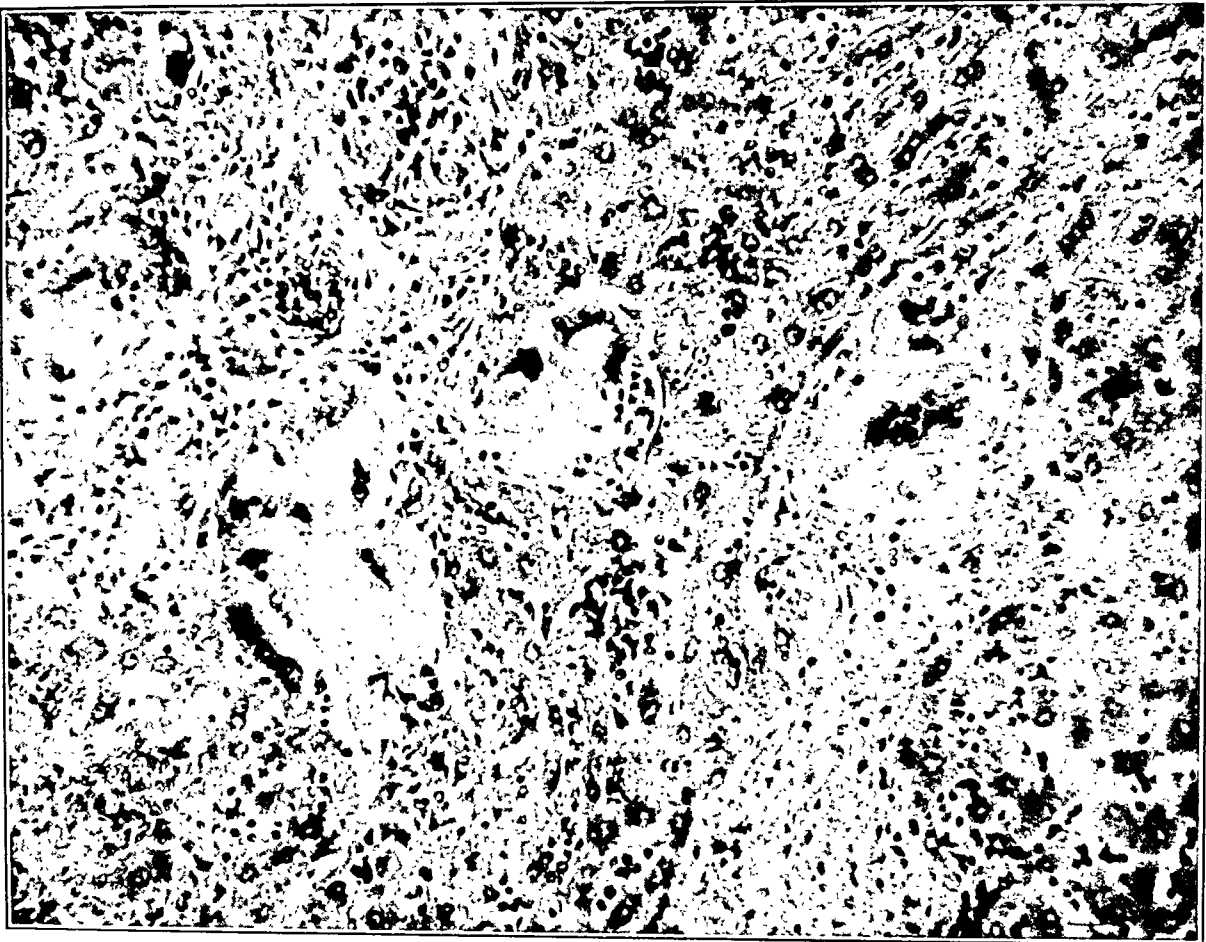


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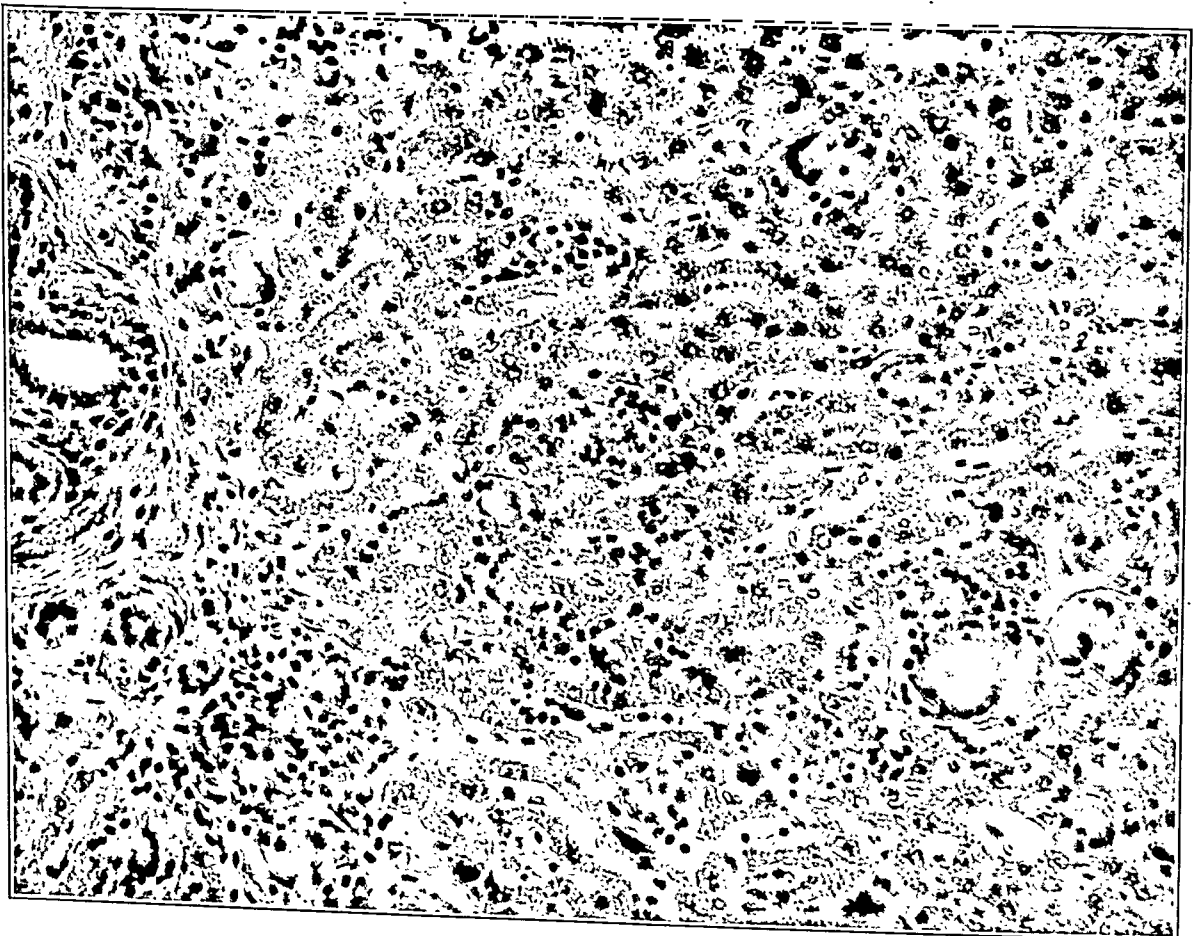
PLATE 86

FIG. 15. Liver (No. 58). Lymphoid, monocytic and giant cells in the sinusoids. Eosin-methylene blue.  $\times 250$ .

FIG. 16. Liver (7 daily intravenous injections of collargol). Pigmented mononuclear and giant cells in the sinusoids. Hematoxylin-eosin.  $\times 250$ .



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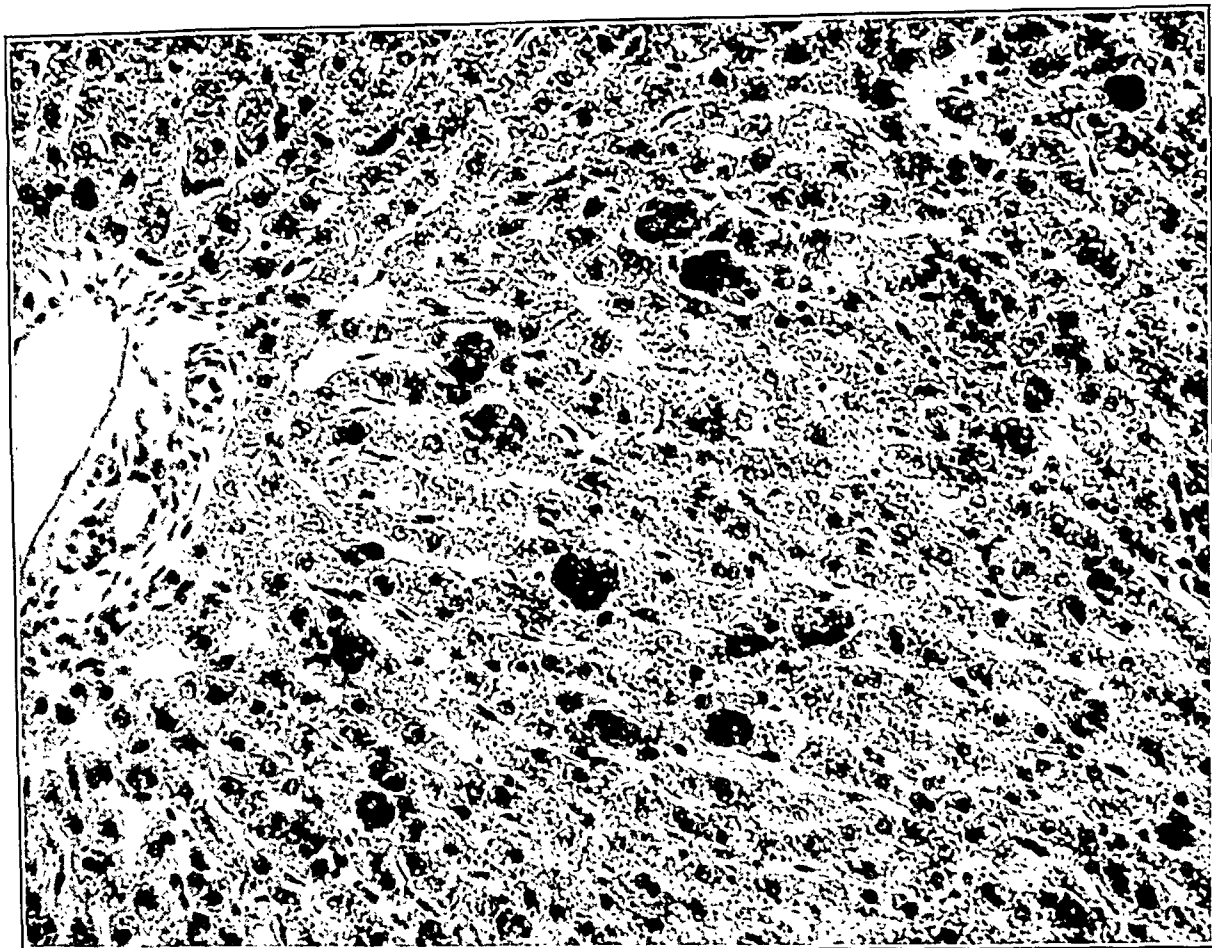
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PLATE 87

FIG. 17. Spleen (No. 34). Two foci, the one composed of monocytes, the other of giant cells. Eosin-methylene blue.  $\times 200$ .

FIG. 18. Kidney (No. 26). Giant cell in a glomerulus. Eosin-methylene blue.  $\times 1000$ .



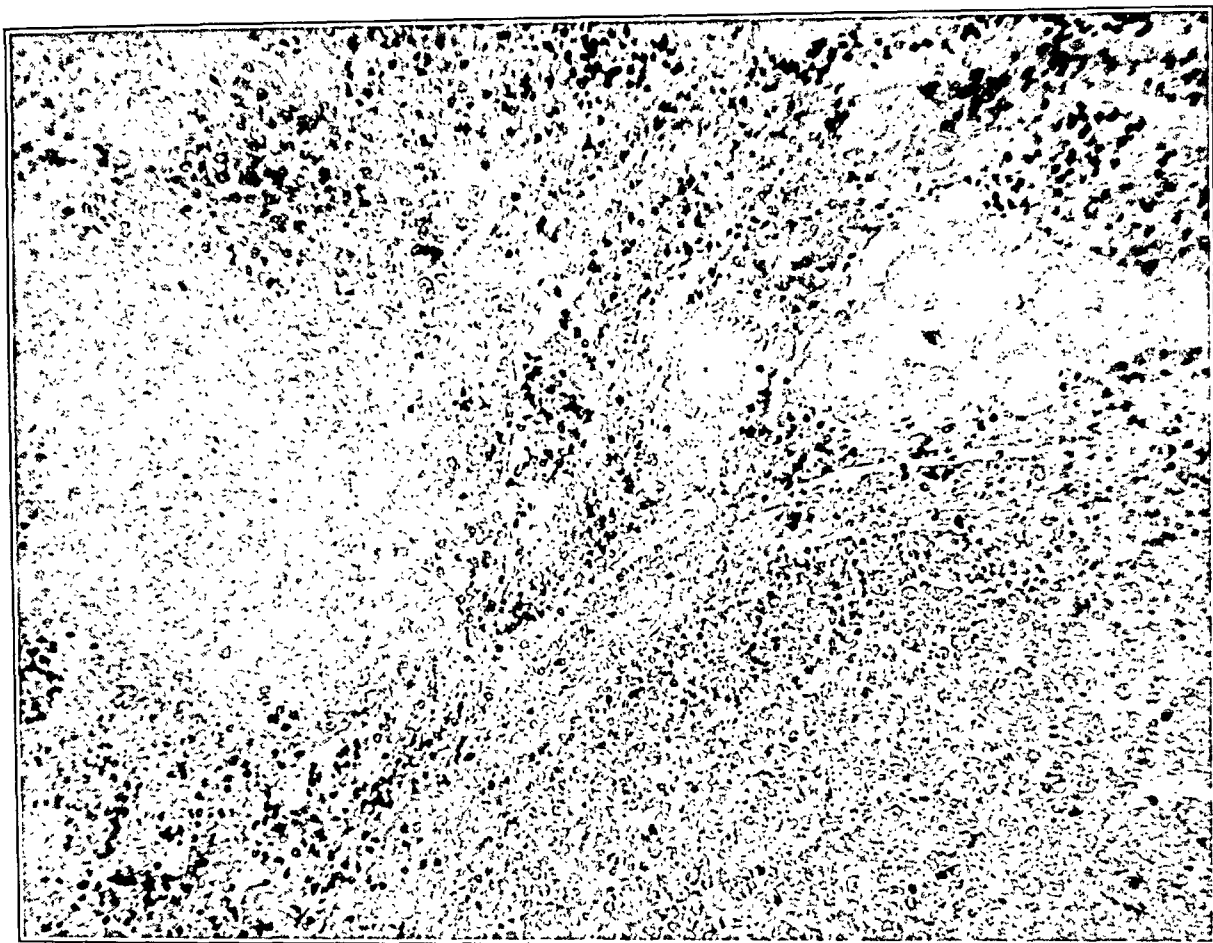
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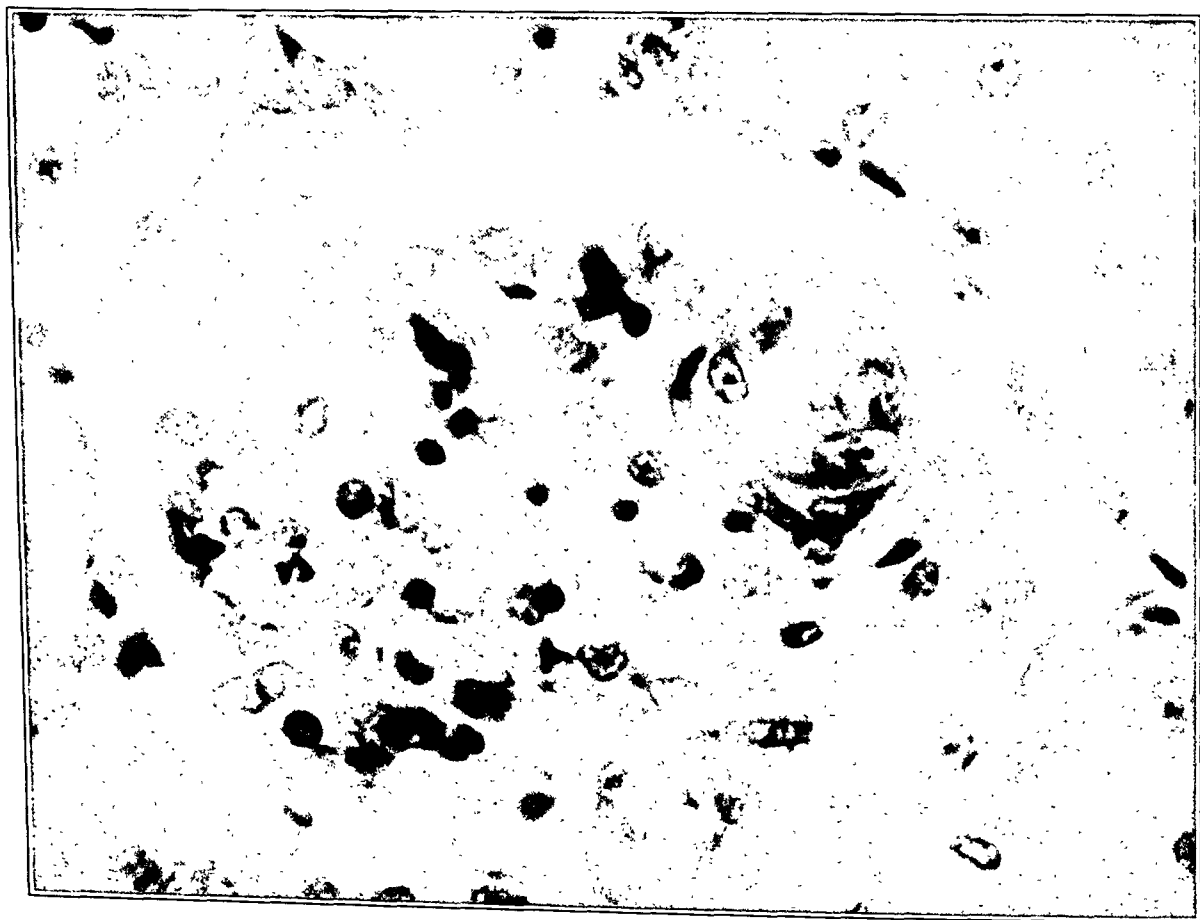
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PLATE 88

- FIG. 19. Adrenal (No. 26). Giant cell in a sinusoid of the *zone fasciculata*.  
Eosin-methylene blue.  $\times 2000$ .
- FIG. 20. Omentum (No. 39). Granulation tissue with two large giant cells.  
Eosin-methylene blue.  $\times 500$ .

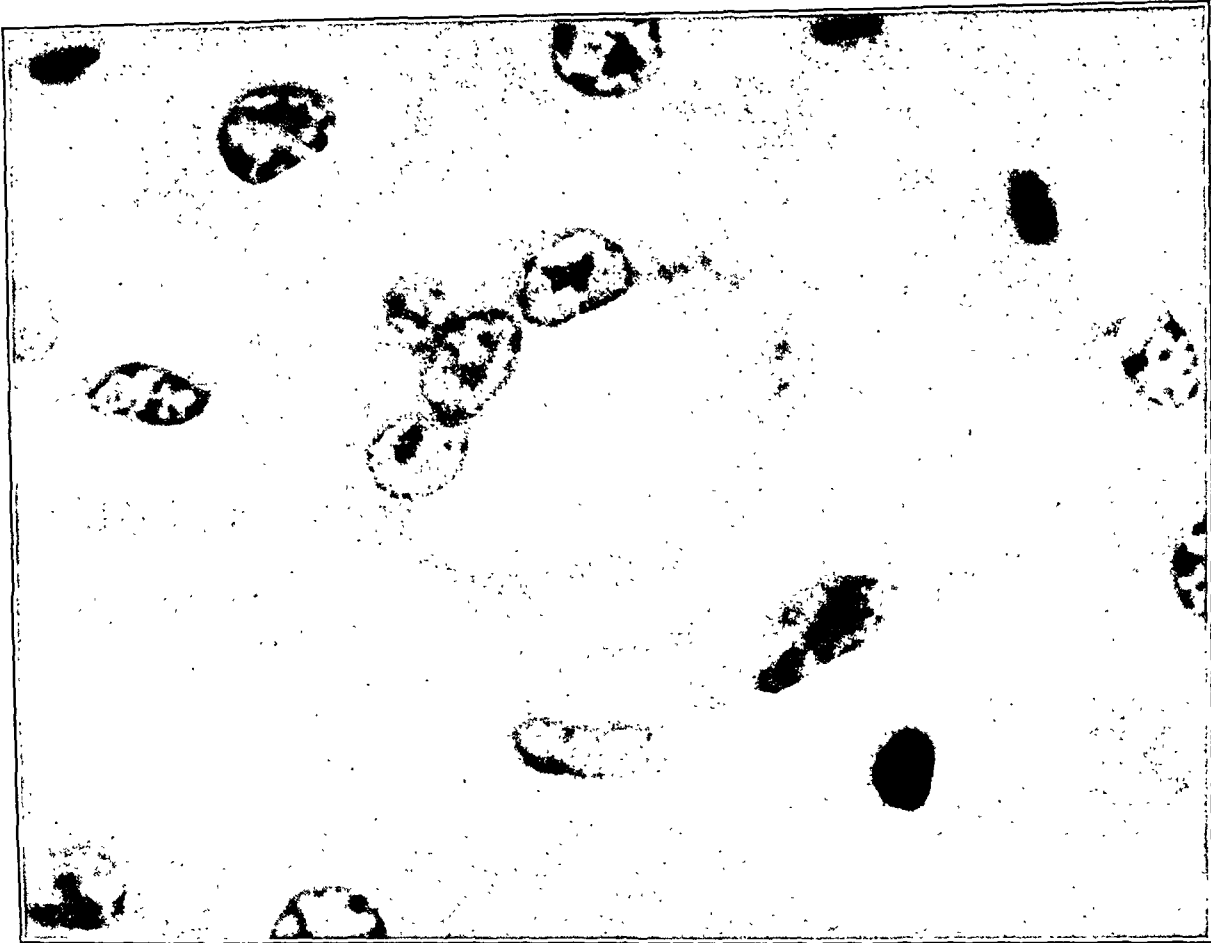


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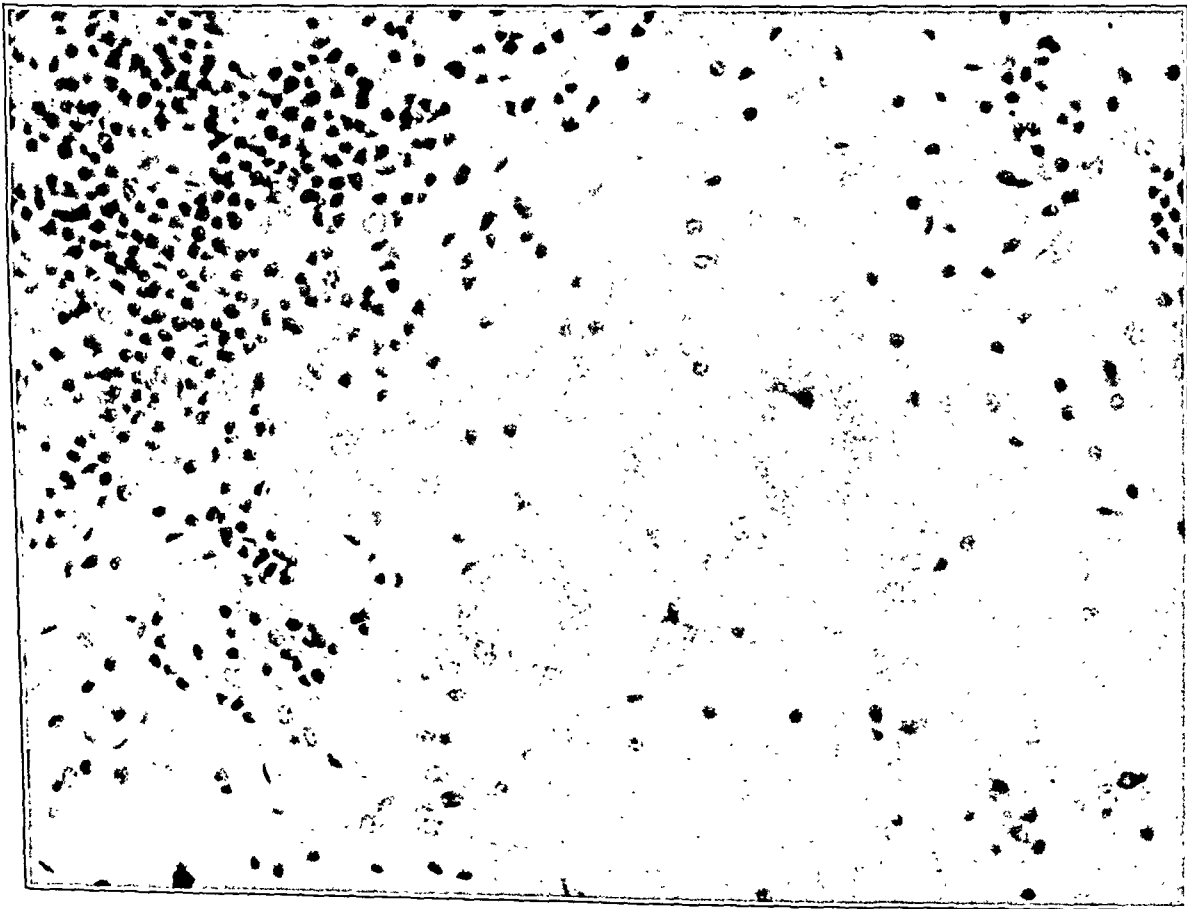


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nervous system. The study of this case has made it possible to complete the pathological picture of the disease, and to describe a new clinical syndrome caused by histologically healed periarteritis nodosa.

Periarteritis nodosa is an inflammatory disease of the arterial system, probably caused by a filterable virus, and characterized by necrosis of the media with fibrinous exudation. This is soon followed by a marked cellular infiltration and granulation tissue formation in and about the arterial wall, together with varying degrees of intima proliferation. The chief secondary changes in the arteries are aneurysm formation, thrombosis and hemorrhage. Death is usually due to hemorrhage from rupture of an aneurysm, or to necrosis or insufficiency of vital organs resulting from thrombosis of the arteries. The veins are free from changes in almost all the cases studied.

At present we know of no predisposing causes of this disease. There seems to be no relation to occupation. However, the disease is four times as frequent in males as in females. It may occur at any age: the youngest patient reported was an infant of three months, the oldest 78 years. About 50 per cent of the cases are found between the ages of 20 and 40 years. The duration of the illness is usually a few weeks to six months, rarely longer than a year. Fishberg's case was ill only six days. Our case of histologically healed periarteritis nodosa lived four years after his one and only attack of acute illness.

Our knowledge of the early changes in the disease is now quite complete except for the question of involvement of the endothelium of the affected arteries. The endothelial changes are difficult to demonstrate, but we believe that they do occur. We know that the most marked changes develop in the media, and this fact has led to the question whether periarteritis nodosa is not related to the changes found in the arteries by Wiesel, von Wiesner, and others in various infectious diseases. They have found degenerative changes in the media which they believe are related to the development of arteriosclerosis in some arteries, such as the coronary. Recently Pappenheimer and VonGlahn have found similar changes in the arteries in rheumatic fever. Spiro has attempted to bring these changes in close relation to those found in periarteritis nodosa, and considers the latter no disease *sui generis*, but only a form of postinfectious mesarteritis. Gruber is also of the opinion that peri-

# A CLINICAL AND PATHOLOGICAL STUDY OF PERIARTERITIS NODOSA \*

## A REPORT OF FIVE CASES, ONE HISTOLOGICALLY HEALED

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### INTRODUCTION

Periarteritis nodosa was first described by Kussmaul and Maier sixty years ago as a disease characterized by the formation of multiple circumscribed nodular thickenings of the smaller arteries of various organs of the body. Since their classic description in 1866 about 150 cases have appeared in the literature. Several investigators have attempted to discover the etiology of this very interesting disease, and to determine the site of origin in the arterial wall. A specific microörganism has not yet been found, but we shall see that the evidence today is in favor of the specific infectious nature of the disease. A very acute case with death in a few days, published by Fishberg, has shown that the primary changes are usually in the inner media and not in the adventitia. The confusion which exists in the literature regarding the site of origin in the arterial wall is due to the fact that changes of different age occur in arteries of the same patient, and often in the same organ. Each acute exacerbation of the illness is accompanied by fresh changes somewhere in the body, and by new symptoms which vary with the localization of the arterial changes.

We shall attempt to summarize our present knowledge of periarteritis nodosa, and to divide the disease into four stages with a discussion of the pathology and clinical symptoms of each stage. This division is based upon a clinical and pathological study of five cases at the First Medical Clinic (Professor K. F. Wenckebach) and the Pathological Institute (Professor R. Maresch) of the University of Vienna. In addition, we had the opportunity of studying three more cases postmortem. One of our cases reveals the histologically healed end-stage involving every organ of the body except the central

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a swelling of the muscle cells with separation by the exudate. *In the smaller arterioles*, those without vasa vasorum, the changes are chiefly in the innermost media, in the subendothelium. No doubt the causative agent enters from the lumen producing endothelial changes as well. A part or the entire circumference of the vessel wall undergoes a coagulation necrosis with a hyaline-like appearance of the inner media. The endothelium may also be affected so that the cells become desquamated or disappear over the affected area. The subintimal changes, the edema and fibrinous exudation may elevate the endothelium causing it to bulge into the lumen or even reducing it to a narrow slit. The fibrin network may extend through the endothelium into the vessel wall. *In the larger arteries*, those with vasa vasorum, the changes appear more often in the outer media, lying near the elastica externa. Here the hyaline areas of necrosis appear and seem to go out from the vasa vasorum or from smaller branches of the vessel. Of course, when the necrotic changes become more extensive they may also reach the endothelium in these vessels. Usually a portion of the vessel circumference presents in the media an area of necrosis with edema and fibrinous exudation. Serial sections often show this region to extend longitudinally in the vessel wall. The areas form an ellipse with the long diameter axially arranged, less often transversely. The entire thickness of the media may become hyalinized. Leukocytes begin to wander into the necrotic area. They are chiefly polymorphonuclear neutrophils, though eosinophiles may also be present in considerable numbers. In this stage the adventitia is often unaffected.

In the early stage changes may also be present in the large arteries and even in the aorta. We have found in one case fragmentation of the elastica interna, with vacuolization and round cell infiltration about the elastica interna in large arteries. That endothelial changes occur in periarteritis nodosa is shown by findings in the brain in one of my cases. Here we observed circumscribed hemorrhages forming a ring about a number of small arterioles, without any demonstrable change in the vessel wall. We must assume, therefore, an increased permeability of the endothelium with hemorrhage by diapedesis into the surrounding lymph space.

*Clinical Symptoms:* In this beginning stage the disease is often latent, especially when the changes are limited to a small area, hence the difficulty of determining the exact age of changes seen post-

arteritis nodosa is a disease of toxi-infectious origin without a specific cause. We cannot share this view, and we believe from our study of five cases of this disease that we are dealing here with a specific infectious disease, the virus of which has an elective affinity for the arterial system and enters the arterial walls directly from the lumen and through the vasa vasorum in the larger arteries.

The view that syphilis is the cause can now be dropped entirely. To be sure, a luetic patient may also contract periarteritis nodosa, but even the cases with a positive Wassermann have shown changes characteristic of periarteritis nodosa and not of luetic arterial disease. Most cases have a negative Wassermann, spirochetes have never been found in the lesions, gummas are not found, giant cells very rarely occur, and the localization is different from that of syphilis. Also the occurrence of periarteritis nodosa in lower animals speaks against syphilis as a cause. The view of several early writers that it attacks individuals with a hereditary or acquired weakness of the arterial walls need no longer be considered.

The most promising work on the etiology of the disease is that of Harris and Friedrichs, and of von Haun, who have succeeded in producing quite similar changes in experimental animals. Furthermore, the finding of a similar disease in lower animals (calf, swine, dog, deer), where it at times occurs in epidemic form, also speaks for the specific infectious nature of the disease.

The organs most frequently attacked are the kidneys (80 per cent), heart (70 per cent), liver (65 per cent), gastro-intestinal tract (50 per cent), pancreas (25 per cent), mesenteric artery (30 per cent), muscles (30 per cent), and peripheral nerves (20 per cent). *The central nervous system is involved* in only 8 per cent of the cases. The disease may also remain confined to a single organ for some time.

We shall divide the disease into four stages according to the changes present in the arteries, and discuss the clinical and pathological findings in each. These are: (1) alterative-degenerative or beginning stage; (2) acute inflammatory stage; (3) granulation tissue stage, and (4) healed end-stage, or scar tissue stage.

#### FIRST OR DEGENERATIVE STAGE

*Pathology:* The beginning stage is characterized by alterative-degenerative changes in the media, with edema and the appearance of a thready fibrinous exudate about the elastica interna. There is

tinuous or intermittent. After a time a secondary anemia develops. The symptoms vary with the location of the process and the nature of the secondary changes in the vessels. Renal, cardiac, peripheral nerve, and gastro-intestinal symptoms are the most common. Icterus may also develop due to involvement of the liver. The spleen is often, though not always, enlarged. There are frequently somewhat enlarged, hard lymph glands. The pulse is as a rule regular and accelerated even when the fever has disappeared.

Death is frequent in this stage, due to rupture of an aneurysm with fatal hemorrhage. This may occur in any of the affected organs. Or, renal insufficiency due to extensive infarction may cause death. Another common cause is cardiac failure due to extensive coronary involvement with thrombosis or intima proliferation.

The finding of subcutaneous nodules, which on histological examination show the characteristic arterial changes, offers a means of making a sure diagnosis *in vivo*.

### THIRD OR GRANULATION TISSUE STAGE

*Pathology:* This is the reparative or granulation tissue stage. There is a marked proliferation of fibroblasts from the adventitia into the inflammatory zone, accompanied by a reduction in the polymorphonuclear leukocytes with an increase in the lymphocytes and plasma cells. Sometimes eosinophiles appear in considerable number. The fibrin network and hyalinized necrotic media are gradually replaced by the cellular granulation tissue rich in fibroblasts and newly formed blood capillaries. This granulation tissue not only replaces the destroyed media, but also extends outward through the adventitia, and longitudinally as well as circularly beyond the area of destruction. It may also penetrate the subendothelial tissue through the defects in the elastica interna. It may even pass through the entire wall and invade thrombi formed in the lumen.

In addition to the granulation tissue formation there is usually a very marked reactive intima proliferation with a partial or total occlusion of the lumen. This intimal thickening consisting of a loose fibroblastic connective tissue usually extends beyond the area of destruction of the media. It is often circular, but may also extend longitudinally on only one side of the vessel. The proliferation is

mortem. At this stage the diagnosis is not possible, the changes are still microscopic. There may be no symptoms whatever; or only a rise of temperature. We hardly need state that we cannot sharply separate these stages from one another. The earliest case in the literature, that of Fishberg, with clinical symptoms of only six days duration, died of renal insufficiency due to extensive infarction of both kidneys. Fever, hematuria, icterus, myocardial insufficiency or pains in the extremities often mark the onset of the disease.

### SECOND OR ACUTE INFLAMMATORY STAGE

*Pathology:* This is the exudative inflammatory stage. A great infiltration of the media and adventitia with polymorphonuclear neutrophiles, sometimes also many eosinophiles, lymphocytes and plasma cells rapidly takes place. The fibrinous exudate extends to the intima and outward in the adventitia. There is destruction of the inner media and of the elastica interna which becomes stretched and fragmented. The process may also extend through the adventitia and spread by way of the perivascular lymphatics. The perivascular connective tissue becomes edematous and leukocytes appear in large numbers. There may be destruction of the entire vessel wall over a part or all of its circumference. The muscle cells of the media become separated from one another and then undergo complete necrosis, the elastica becomes fragmented, survives longer than the muscle cells, but soon also disappears. When the exudate reaches the intima the fibrin threads may penetrate into the lumen and leukocytes wander from the lumen into the subendothelial tissue. A marked subendothelial connective tissue proliferation takes place, a reactive intima proliferation. At the height of this stage secondary thrombosis of the lumen with infarction of the various organs is common. Toward the end of this stage aneurysm formation or rupture of the vessel wall with hemorrhage into the adventitia or surrounding tissue occurs. There is no suppuration, and pyogenic bacteria are not found. In cases without aneurysm or nodule formation the changes may be overlooked without microscopic examination.

*Clinical Symptoms:* This is the stage with high fever, chills, a polymorphonuclear leukocytosis (sometimes also an eosinophilia) and all the symptoms of a severe infection. The fever may be con-

narrow channels run axially through the greatly thickened vessels. Nodular thickenings of the outer wall occur when the process involves the outer media and adventitia. Then a marked periarterial fibrosis takes place as a result of the histological healing of the granulation tissue in the adventitia and surrounding connective tissue.

Three processes here are active, separately or very often combined: (1) a marked subendothelial connective tissue proliferation which may be accompanied by the new formation of elastic fibrils and often extends beyond the area of media destruction; (2) thrombosis of the injured vessels with complete organization with or without recanalization, and (3) healed granulation tissue scar formation in and about the injured vessel wall. These three processes can often, but not always, be distinguished from one another.

Only cases of severe periarteritis nodosa with a tendency toward thrombosis and intimal proliferation will reach this advanced stage. The cases with aneurysm formation usually lead to fatal hemorrhage. Also, early complete occlusion of the blood supply to vital organs, whether by thrombosis or intimal proliferation or both, will result in early death. It is, therefore, possible for a generalized periarteritis nodosa to become histologically healed only when the blood supply is not reduced below the minimum necessary for the maintenance of function of the vital organs. Case 5 will demonstrate this fact very well. No disease better illustrates the great factor of safety in the blood supply to vital organs than does periarteritis nodosa.

The intimal proliferation is a reactive local proliferation in response to the injury of the inner media. It is characterized by the absence of blood capillaries and hemosiderin deposits such as are seen in organized granulation tissue or thrombosis. The fibers are arranged more or less concentrically in cross-sections of the artery. The elastica interna presents all stages of degeneration and necrosis. A new formation of elastic fibrils with marked thickening of the intima occurs in arteries where the changes in the media are less severe and hence the elastica has not been destroyed.

The healed granulation tissue which grows into the adventitia and media is characterized by a richness in fibroblasts, the presence of numerous newly formed blood capillaries, and fine deposits of hemosiderin. As this tissue grows older it becomes more and more hyaline and fibrous, the capillaries are compressed and obliterated,

usually thickest at the site of the lesion with the result that the narrowed lumen often lies eccentrically on the less affected or unaffected side. The spread of the intima proliferation beyond the area of media destruction explains why we so often find in transverse sections a marked intimal thickening without changes in the vessel wall. We have convinced ourselves by a study of many serial sections from different organs that these areas lead to vessel wall changes usually at the place where the intimal proliferation is most marked. In other words, the intimal thickening in periarteritis nodosa is usually the consequence of vessel wall changes in the media.

In this stage of granulation tissue formation, aneurysms or rupture of the wall may occur or there may be only a thickening of the wall from intimal proliferation, or granulation tissue formation outside the adventitia. Cases without nodule formation on the vessels may be overlooked unless examined microscopically.

*Clinical Symptoms:* In this stage marked anemia, emaciation and marasmus usually develop if the involvement is widespread. If confined to a non-vital organ or tissue, healing may occur, clinical as well as histological. The fever and leukocytosis usually drop or may entirely disappear. However, acute exacerbations are the rule, with the development of fresh foci. The symptoms in this stage are due to vascular occlusion of the kidneys, heart, gastro-intestinal tract, peripheral nerves, muscles, and glands of internal secretion. They are hypertension, nephritis, renal insufficiency, cardiac failure, intense pains in the abdomen, icterus, ulcerations or gangrene of the bowel, peripheral neuritis, muscular atrophy, Addisonoid symptoms, etc. Or a sudden collapse due to internal hemorrhage may occur (kidneys, liver, gall bladder, gastro-intestinal tract, pancreas, lungs, brain, etc.). The diagnosis of rupture of an artery can then be made. Such hemorrhage is more common in the second stage of the disease.

#### FOURTH OR HEALED GRANULATION TISSUE STAGE

*Pathology:* This is the histologically healed end-stage or scar tissue stage, as illustrated by the case of periarteritis obsoleta nodosa of four years duration, which we shall describe. The destroyed arterial wall, often only the inner media including the elastica interna, is replaced by an indifferent fibrous scar tissue poor in nuclei. The lumen is greatly reduced in size or totally obliterated. Often only

## CASE REPORTS

**CASE 1. *Clinical History:*** The patient, Franciska H., a dressmaker 46 years of age had the following history: Both parents died of old age. The patient has four sisters, one has heart disease, another gastric ulcer. The patient was always well until three years before her present illness when she had pneumonia. A year ago she had a "rheumatism" confined to the left shoulder joint.

The present illness began six weeks before entrance into the hospital with intense pain in the leg muscles so that the patient couldn't walk. Soon similar pains developed in the arms and hands. She stated that she had no fever at this time. About fourteen days ago the patient suddenly developed high fever to 40° C, with chills and slight sore throat. The angina disappeared after a few days, but not the fever.

Examination showed a medium-sized woman with poor musculature and very little subcutaneous fat. The cranial nerves were all free from disturbance. In the chest there was a slight dullness over both apices, but the lung borders were normal. The heart was not enlarged. There were no murmurs. The liver was slightly enlarged on percussion. The spleen was not palpable.

The pulse rate was 100-120 but regular.

There was marked tenderness on pressure over the sciatic nerve, also the peripheral nerves of the upper extremities. The patellar reflex was present, the Achilles' reduced. There was no Babinski, no clonus.

The white blood count was 12,400. The Wassermann was negative. Blood cultures taken at the height of the fever were negative. Repeated examination of the sputum failed to reveal tubercle bacilli. The patient's septic temperature continued unchanged in spite of the use of aspirin, electrocollargol, Pregl's solution, etc. The heart action remained good and no signs of endocarditis could be found.

Twelve days after entrance, on October 24, the pains in the arms increased. There were sensory disturbances in the radial nerve distribution of the right hand. The reflexes in the left arm were increased. On November 3, the condition of wrist-drop developed on both sides. The radialis no longer reacted to galvanization or faradization.

On November 5, the findings in the arms were as follows:

*Right Arm:* The patient could carry out all movements in the shoulder joint and elbow, but only slowly and with effort. The movement of the wrist and finger joints was practically impossible. The radialis showed no reaction to faradic or galvanic current. The ulnaris reacted slightly to faradization.

*Left Arm:* There was slight improvement, the radialis and ulnaris reacting to faradic with slow contraction of the muscles.

*Right Leg and Left Leg:* The peronealis reacted but the tibialis did n't.

On November 12, the reflexes were absent in the lower extremities. The reaction of the nerves of the arms varied, one day they reacted slightly, the next day not at all.

The patient became weaker every day and drugs were ineffective. On November 19, the patient collapsed and received camphor and caffeine. On November 28, the patient developed severe pain in the abdomen; the large intestine could be palpated and was strongly contracted. There was a slight diarrhea with bloody stool. On November 29, the patient became comatose, and developed a right-sided facial paralysis. Speaking was difficult, also swallowing. The patient died on November 30, in deep coma.

and the hemosiderin slowly disappears. The healed scar tissue remains as evidence of the severity and extent of the earlier acute inflammatory process. The perivascular mantles of scar tissue, which we have found surrounding the arteries with extensive destruction, we consider to be characteristic for this healed end-stage. These arteries lie embedded in thick sheaths of scar tissue radiating through the affected organ, producing the appearance of an interstitial scar tissue formation. Where the arterial destruction is greatest there the periarterial healed granulation tissue is the thickest.

The final organ changes in this stage are contracted kidney, contracted scarred liver (*hepar lobatum*), myomalacia scars, adrenal atrophy, necrosis or ulceration in the gastro-intestinal tract, encephalomalacia, muscle atrophy, and peripheral nerve degeneration. In other words, we may have healed infarcts or atrophy in any of the organs with characteristic arterial changes. The vessel changes may be microscopic or macroscopic. With severe changes there are often nodules on the arteries produced by healed aneurysms or periarterial scar tissue formation. Elastic tissue stains of the arteries, and serial sections should be made in all suspected cases.

*Clinical Symptoms:* There is an absence of fever in this end-stage when, as in my case, all the lesions in the body are histologically healed. The pulse remains accelerated, but is usually regular. The leukocyte count is normal. The symptoms are due to a progressive reduction of the blood supply in the various organs, and may be as variable as in the earlier stages, depending upon the localization of the vascular process. We can expect as most common: renal insufficiency, cardiac failure (without pulmonary or other demonstrable cause) which is resistant to the action of digitalis, degenerative polyneuritis, marasmus, muscular atrophy, abdominal cramps, *hepar lobatum*, gastro-intestinal ulceration, encephalomalacia, adrenal insufficiency or even polyglandular insufficiency.

The difficulty in the diagnosis of the disease in the earlier stages seems to be even greater in this histologically healed end-stage. The history of a previous severe febrile attack, the symptoms of renal involvement, polyneuritis and polymyositis, and abdominal pains, that most common tetrad of symptoms in periarteritis nodosa, might enable one to diagnose the healed end-stage. The finding of nodules in the skin with the characteristic histological changes would render possible the diagnosis.



Summarizing our observations in this case we find:

A woman of 46 years developed a severe polyneuritis with pain, marked weakness, and atrophy of the muscles of the extremities, loss of reflexes and wrist-drop. This was accompanied by a persistent high septic temperature, a rapid regular pulse. Then came a severe collapse, followed by intense abdominal pain and bloody diarrhea. Death was due to cerebral apoplexy with rupture into the lateral ventricle. The autopsy revealed a generalized periarteritis nodosa. This variety of symptoms: weakness, septic temperature, polyneuritis, abdominal pain followed by melena, and cerebral hemorrhage, could not be "brought under one hat" by the clinician. We want to emphasize this very fact as characteristic of most cases of periarteritis nodosa. When we find such a variety of symptoms, referable to various organ systems, we should think of a common vascular cause such as periarteritis nodosa.

CASE 2. *Clinical History:* Joseph H., aged 55 years, had always been well. The family history revealed nothing of importance. Except for a severe burn sustained by the patient fifteen years ago he had always been well. The patient entered the hospital on October 20, 1925.

The present illness began three weeks ago with intense pain in the right calf of the leg. The pain then spread to the knee region and the toes. He had the feeling that the leg was swollen. The pain was more severe on walking than on lying in bed. There was a sensation of numbness in the leg, with paresthesia at times. A week later the same symptoms developed in the left leg, but not so severe as in the right. Still he could continue his work until October 16, four days before entrance into the hospital. On October 17, the pains became so intense that the patient was forced to bed. At this time similar symptoms developed in the left forearm and hand. The left extremity was weaker and anesthetic. The patient also had been vomiting two or three times a week in the morning for five weeks.

The patient drank five to six beers and about one quarter of a liter of wine daily. He had been a heavier drinker. Venereal disease was denied. The patient smoked, but not to excess.

We shall not give the complete physical findings, but only those facts of interest in connection with the disease.

The patient was a medium-sized well developed man in good nutritional condition. There was no edema, icterus or cyanosis. The head was entirely normal, the pupils reacted normally to light and accommodation. The thyroid was not enlarged, there were no abnormal glands palpable.

The heart and lungs were normal, except for a few râles at the base of the right lung posteriorly. The pulse was 100, regular. The temperature of the patient was remittent, rising afternoons to as high as 39° C. The liver and spleen were not enlarged. There was no abnormal resistance in the abdomen. The blood pressure was 145 to 160 mm. systolic. The Wassermann test of the blood was negative.

The movement of the left arm and both legs was considerably reduced, as also

The clinical diagnosis was infiltration of the right upper lobe with abscess formation, polyneuritis, metastatic process in cerebro, enteritis.

The autopsy, performed by Dr. Feller, revealed an extensive periarteritis nodosa involving almost all the organs of the body, including the central nervous system as well as the peripheral nerves. Most marked are the changes in the gastro-intestinal tract. In the stomach there are infiltrations on the arteries up to the size of a pea along the greater and lesser curvatures. The nodules in large numbers produce protuberances of the mucosa so that the inner surface of the stomach appears nodular. Especially numerous are the nodules about the small arteries at the mesenteric attachment of the small and large intestine. Also in the intestines the nodules often cause protrusion of the mucosa into the lumen. In places the intestinal wall appears to be undergoing necrosis. In the large intestine (cecum, ascending and transverse colon) are a number of bleeding ulcers of the mucosa, some covered with a necrotic membrane. These vary in size up to 2 cm. In the ileum longer stretches of the wall are necrotic. There is a circumscribed fibrinous peritonitis over these areas.

Miliary to pea-sized nodules are found on the peripheral arteries. In the extremities along the muscle and nerve branches are numerous pinhead to millet-seed-sized nodules. Many nodules are seen in the liver along the branches of the hepatic artery, in the kidneys and in the pancreas. There are many nodules and areas of thickening on the coronary arteries.

A large fresh cerebral apoplexy exists in the region of the left basal ganglia and reaching almost to the cortex, with perforation into the left lateral ventricle. A confluent lobular pneumonia is present in the right lung, with fibrinous pleuritis.

Histological studies showed acute and chronic changes in the arteries of every organ in the body. I wish to call attention to the presence of multiple small periarteriolar hemorrhages in the brain, in some places without demonstrable arteriolar change other than a slight swelling or edema of the wall. The very extensive degeneration of the peripheral nerves, always accompanied by severe arterial changes with obliteration of the lumen in many places, is of special interest in this case. Also the marked involvement of the gastro-intestinal tract with numerous nodules in the submucosa commands our attention.

Hydrothorax and hydroperitoneum are present. Hemorrhagic infarcts are visible in both lungs, with a lobular pneumonia and fibrinous pleuritis in the lower lobes. The heart is enlarged, with eccentric hypertrophy of both ventricles, and dilated auricles. The valves are all normal. The myocardium is pale grayish red to yellow, and its consistency is reduced. Everywhere are small scars of whitish color in addition to the general fatty degeneration. The coronaries are straight and delicate, but along their course are numerous pin-head-sized whitish spots or nodules.

The aorta is smooth and appears entirely normal, as also its large branches. The thyroid appears normal. The liver is large, of normal consistency. On the surface are many irregularly outlined bluish red depressions 1 to 3 cm. in diameter and easily visible through the delicate capsule.

These areas are mostly in the left lobe and left half of the right lobe. On the cut surface they are dark red and depressed. The liver parenchyma is destroyed leaving behind a vascular network filled with blood. Here and there are thickened vessels with narrow or obliterated lumen.

The gall bladder appears normal. The spleen is enlarged. The kidneys are large, the capsule adherent and thickened. The surface is very irregular with numerous dark red retractions of various sizes. There are other small yellowish necrotic areas. On the cut surface the thickened arteries are visible, some appearing as round grayish areas without any lumen. The adrenal and pancreas appear normal.

On the arteries along the lesser curvature of the stomach, on the arteries of the small intestine in the immediate vicinity of the mesenteric attachment to the bowel, and on the vessels from the mesocolon to the large intestine are everywhere numerous nodules, often arranged in chains like a string of pearls (*perlschnurartig*). The nodules are mostly of the size of a hempseed. Some vessels present thickenings in the wall which hardly protrude beyond the surface. The mesenteric lymph nodes are somewhat enlarged. The prostate, testicles and epididymes appear normal. There are fresh thrombi in the veins of the prostatic plexus, in the posterior tibials and the muscle branches of the lower extremities.

In this case of acute periarteritis nodosa we find as the predominating symptom the polyneuritis, with paresthesias, muscle pains and weakness, anesthesia, loss of reflexes and wrist-drop. The

the strength in these extremities. The patient dragged his foot somewhat on walking. The patellar and Achilles' reflexes were somewhat reduced.

The patient perspired considerably. He developed paresthesias in the right hand on October 24. On October 27, the pain in the calves disappeared, and the paresthesia was less marked. The patellar and Achilles' reflexes were gone. There was a slight edema over the internal and external malleoli. The muscle sense of the fingers and wrist was greatly disturbed. The right hand developed a wrist-drop with hyperesthesia in the radial region. There was a marked atrophy of the interossei muscles.

On November 3, the edema about the ankles was still present. The temperature rose daily to 38 or 39° C. Both arms could be moved only with difficulty. The urine showed a trace of albumin, but no renal elements.

On November 10, the pain in the calves was still present. The heart dullness was enlarged, the pulse 104. On the 15th, the sensory disturbances of the ends of the lower extremities were somewhat reduced. The patient could lift his right foot a little better. On November 20, the movement of the wrists and finger joints was somewhat better.

On December 2, the patient developed severe dyspnea, with numerous bronchial râles over the entire lung. He had a tachycardia. The liver was enlarged, hard and painful to pressure. There was no ascites. On December 7, dullness was found over the right base posteriorly. The heart apex lay in the 6th interspace in the anterior axillary line. The edema of the lower extremities and sacral region was increased.

The patient died on December 15, after an illness of about ten weeks. The clinical diagnosis of Professor H. Schlesinger was: polyneuritis alcoholica, myodegeneratio cordis with marked decompensation, pneumonia.

The following laboratory findings are also of interest:

October 20: Urine negative, specific gravity 1020.

November 27: Urine: albumin positive, blood positive with numerous red corpuscles, few leukocytes and epithelial cells.

November 30: Urine: albumin positive, with few granular casts, leukocytes and epithelial cells, but no red corpuscles.

December 7: Albumin negative, no red corpuscles.

December 13: Albumin positive, urobilin and urobilinogen positive.

December 14: White blood count .....	17,500
Polymorphonuclears .....	81
Monocytes .....	5
Lymphocytes .....	14

The autopsy on December 15, 1925, performed by Dr. Matras in the Pathological Institute of Professor Maresch, revealed the following:

There is a universal edema of the skin. The dura of the brain is tense, the meninges are somewhat thickened.

The fluid in the subarachnoid space is increased, with a slight edema of the brain. The ventricles are slightly enlarged and contain a clear fluid. The cord presents no macroscopic changes.

Six days ante mortem the patient had a collapse, the pulse being 140, the temperature only 36.8° C. The next day râles were heard at the base of both lungs. Then dullness developed on both sides. The dyspnea became more severe and bronchial breathing was audible over the entire lung. The patient died on September 17, after a six-day fever-free period. The clinical diagnosis was sepsis of unknown cause with terminal afebrile pneumonia.

The postmortem examination performed by Dr. Feller in the Pathological Institute of Professor Maresch revealed the following:

A recent confluent lobular pneumonia of all the lobes, with a high grade pulmonary edema; eccentric hypertrophy of both ventricles, especially the left. The coronary arteries are normal except for slight atherosclerosis. There is a fatty degeneration of the myocardium. A right-sided aorta, which runs over the right bronchus and behind the esophagus, is found.

The liver displays on its surface numerous irregularly outlined dark red depressions which vary in size up to 2 cm. in diameter. Similar areas can be seen on the cut surface. The branches of the hepatic artery show marked periarteritis nodosa with occlusion of the small branches. The reddish depressed areas represent infarcts.

The kidneys are irregularly coarsely granular. The reddish gray depressions represent multiple healed infarcts. There are also many fresh anemic infarcts present. The branches of the renal artery show extensive changes with wall destruction, aneurysm formation, thickening of intima and thrombosis. Many are in the acute inflammatory stage. Those in the granulation tissue stage are often surrounded by a mantle of periarterial connective tissue. Some of the glomeruli present the typical picture of a glomerulonephritis. The pancreas and adrenal are macroscopically normal. The arteries of the arms and legs show miliary aneurysms on their smaller muscular branches. The arteries of the peripheral nerves show no typical changes macroscopically, but microscopic examination reveals marked changes.

The stomach and intestines appear normal. There are also no changes in the central nervous system.

When we briefly summarize this case we find: A man of 50 years suffered from an angina followed by three attacks of fever. The last attack was accompanied by edema of the legs with intense pain in the muscles, chiefly the peroneal group. He had a polymorphonuclear leukocytosis and intermittent temperature to 39.3° C, a nephritis with hematuria but no increased blood pressure. Death

second most important symptom is the remittent temperature. Vomiting was the only gastric symptom. Then came signs of cardiac decompensation. The renal symptoms were the transient hematuria and albuminuria. Death resulted from cardiac failure with pneumonia and hemorrhagic infarction of the lungs.

The histological changes were typical of acute and subacute periarteritis nodosa of the heart, kidneys, arteries of the peripheral nerves, liver, and mesenteric branches.

CASE 3. *Clinical History:* Hugo H., 50 years old, entered the Wenckebach Clinic on August 19, 1925. The patient had measles, whooping cough, scarlet fever and diphtheria in childhood. At the age of 21 years he had a soft chancre. The patient denied the use of alcohol and was a moderate smoker.

The present illness began in June, about two months before his entrance into the hospital. He had an attack of angina with fever lasting ten days. He remained in bed three weeks and then returned to his work. After a four-day afebrile period he developed a second attack of fever lasting five days. Fourteen days later a third attack occurred. This began about the second of August and lasted until his admittance on August 19. His temperature during the attacks reached 39 to 39.5° C. During the third attack *pain and swelling of the legs appeared*. A week's rest in bed brought relief. *The pain was chiefly in the calves and in the peroneal muscles, making walking impossible.*

The examination on August 20, revealed the following:

A well developed man, with somewhat atrophic musculature. The temperature 37.7° C, respiration 20, pulse 90 and regular. The pupils were equal, reacted normally to light and accommodation. The tonsils were enlarged. The lungs presented nothing pathological, except for a dullness over the left apex. The heart was slightly enlarged to the left. The blood pressure was 150 systolic. A soft systolic murmur at the apex was heard on August 29, ten days after admission. The liver was slightly enlarged. The spleen was not palpable.

On both legs there were *areas of paresthesia involving chiefly the peroneal distribution*. In the center of these areas there was complete anesthesia. Here the pain was most intense a few weeks earlier.

Examination of the fundus oculi was negative. X-ray examination of the chest showed a darkening of the left apex, with calcified spots in the left hilum region. The heart showed an enlargement of the left ventricle. I found a hitherto undescribed anomaly of the aorta, a right-sided retro-esophageal aorta which was confirmed at autopsy. The blood and spinal fluid Wassermann were negative. Agglutination reactions for typhoid and paratyphoid were negative. The blood examination showed 12,000 leukocytes with 82 per cent polynuclear neutrophils and 3 per cent eosinophiles. The red count was 3,600,000 and the hemoglobin 60 per cent, an index of 0.9. The spinal fluid was normal.

The examination of the patient failed to reveal the cause of his intermittent temperature which in the mornings reached as high as 39.3° C. The patient gradually developed an edema of both legs. The pulse was always rapid, averaging 110.

Several examinations of the urine revealed about 1/4 per 1000 albumin and numerous red blood cells and some leukocytes in the sediment. The residual nitrogen in the blood was normal.

Lumbar puncture yielded a pressure of 300 mm., the spinal fluid clear with 15 cells per cmm. The intense headache persisted. The patient had signs of cardiac failure with edema of the extremities and also râles at the base of both lungs. On December 10, the patient developed a deviation of both eyes to the left, unconsciousness and tracheal râles, and died.

The blood pressure of the patient varied between 170 and 190 systolic, and 110 and 120 diastolic. The concentrating power of the kidneys was reduced, the urine specific gravity never above 1017. The sediment contained red cells, leukocytes and casts, also renal epithelium. Once the stool gave a positive reaction for blood. The blood Wassermann test was negative. The temperature was subnormal mornings and reached 37.5° C afternoons. It was never above 37.5° C during the stay in the hospital.

The autopsy performed on December 10, revealed a chronic periarteritis nodosa affecting chiefly the kidneys, liver, heart, peripheral nerve vessels, and mesenteric arteries. There are numerous nodular thickening of the arteries. The most marked changes are seen in the coronaries, hepatic artery branches, renal arteries and mesenteric arteries.

The myocardium is macroscopically unchanged. There is a marked hypertrophy of the heart, especially the left ventricle. The liver presents numerous smaller and larger depressed and irregularly outlined gray-red areas, which consist almost entirely of blood capillaries and in which the liver parenchyma is destroyed.

The kidneys show the most marked changes. The surface presents relatively small light gray and grayish yellow, flat prominent smooth areas with irregular outline. These represent the rests of the cortex. Between these areas are numerous dark red depressions representing the healed infarcts due to arterial occlusion. The larger branches of the renal artery in the hilum region are greatly thickened. The testicles contain a number of smaller and larger fibrous scars. The vessels of the peripheral nerves *show no macroscopic changes*. The cerebral vessels appear macroscopically unchanged, yet in the region of the left caudate nucleus there are several grayish red unsharply outlined and slightly depressed areas. Also in the cerebellar cortex there are multiple foci of hemorrhage of various size up to 2 cm. Bilateral pleural effusion and uremic pericarditis are also present.

Histologically we find the vascular thickenings to be due to a thickening of all the layers of the wall, especially the intima and adventitia with rich perivascular connective tissue formation. In some places acute inflammatory changes are still present, with

was due to confluent lobular pneumonia and cardiac weakness. The postmortem examination revealed an acute and chronic periarteritis nodosa affecting chiefly the kidneys, liver, muscles of the extremities, and peripheral nerves. The presence of multiple infarcts in the kidneys in the absence of an endocarditis should always call to mind the possibility of periarteritis nodosa. We have diagnosed two cases postmortem by this finding, together with thickenings and nodule formations on the arteries.

**CASE 4. Clinical History:** Jacob S., aged 34 years had no children's diseases, and was never seriously ill until his present illness. The family history revealed nothing of importance. The patient's present illness began in June, 1924, five months before he came to the clinic. He had at the onset *generalized rheumatic symptoms* with temperature to 39° C. *There was no swelling of the joints*, but slight edema of the legs in the afternoon when the patient was up and about. He was under a physician's care and at home for seven weeks. He then went to a clinic where he remained four weeks on account of an acute nephritis and left feeling quite well. The rheumatic pains in the extremities disappeared. A few days after returning home the patient again developed the pains in the extremities with edema of the legs. Then severe headaches set in for about fourteen days. About two weeks after the onset of this attack an orchitis appeared. It lasted about four weeks. Since the development of the headaches the patient's vision has suffered. There were no mental disturbances. On account of the severe headaches the patient came to the clinic on November 17, 1924. The patient stated that he drank about one liter of wine daily, and smoked 30 to 40 cigarettes. He denied venereal infection.

The patient was a medium-sized well nourished man. He was somewhat stuporous. The skin was pale but not edematous. The pulse was 88, regular. The pupils were equal and reacted normally to light and accommodation. The thyroid was not enlarged, nor were any enlarged glands present in the neck region. The thorax was symmetrical, the lung borders normal. The heart dullness was slightly increased to the left. The aortic second sound was accentuated. The liver and spleen were not palpable. There was no ascites. The external genitals appeared normal.

Examination of the eyes on November 18, revealed the following: In the right eye there were no certain changes in the fundus. In the left there were variations in the caliber of the small arteries in the region of the papilla. There was a slight edema of the retina about the papilla. The residual nitrogen in the blood on November 20, was 34 mg. Examination of the urine showed albumin 2 per 1000 (Esbach) with erythrocytes, leukocytes and many granular casts in the sediment.

On November 27, the patient's headaches became more intense, with vomiting, sleeplessness and still greater reduction of vision. On December 3, the fundus showed: In the right eye the border of the papilla was indistinct, with marked edema of the retina, and small hemorrhages. No foci of retinitis were visible. There was an ablatio retinae in the nasal peripheral zone. The left eye showed more marked unsharpness of the papilla and edema around it. Many radiary hemorrhages were seen about the papilla. Ablatio retinae in the lower periphery. The residual nitrogen on December 5, was 50 mg.



tity of urine decreased. The edema of the legs was relieved by puncture but soon recurred. On October 5, the patient became very dyspneic with marked congestion of the veins of the neck. The dullness over both lungs reached to the fourth rib. The patient died on October 11, 1922.

At no time during the patient's stay in the hospital was there any fever, the highest temperature being 36.8° C. At the same time the pulse was always 100 or more.

The following laboratory findings are of interest:

The stomach contents was anacid. The stool was positive for blood. The urine contained 1/4 per 1000 albumin, but no erythrocytes or leukocytes. The specific gravity was low, the chlorides reduced. The Wassermann was negative. The fundus oculi was examined October 7, and reported normal.

The autopsy was performed by Dr. Feller on October 11, 1922. The lungs are free from tuberculosis. There is a marked compression atelectasis of both lower lobes, with slight emphysema of the upper lobes. The lungs are congested. The heart is hypertrophic, especially the left ventricle. The subepicardial fat is absent. The coronary arteries are thickened. Numerous nodular thickenings are seen in the wall of both coronary arteries. On cross-section the wall appears greatly thickened, the lumen reduced to a narrow slit in places.

The aorta is practically free from atherosclerosis. A thrombus is present in the left auricle.

The liver is relatively small, and resembles a *hepar lobatum syphiliticum*. Its surface is coarsely granular, and in a number of places there are deeply penetrating depressed scars. The capsule of the liver is wrinkled in the sunken areas. The left lobe is small and more markedly affected than the right. On the cut surface are found septa of connective tissue containing obliterated thickened branches of the hepatic artery. Nodular thickenings occur on some of the branches. There are also areas of normal liver tissue with normal acinous structure; and immediately adjacent are areas of marked congestion in which the parenchyma has disappeared. The spleen is slightly enlarged. The pancreas is atrophic, but its lobular structure is well preserved.

The kidneys are about normal in size. The arteries are thick-walled and gaping. The surface is very irregular with numerous depressions of various size. These are reddish in color. On the cut surface are partly wedge-shaped and partly more rounded elevations which correspond to the nodules of parenchyma seen on the surface. In the retracted areas the cortex is absent. Everywhere

leukocytes and a few eosinophiles. Many arteries are occluded by thrombi undergoing organization. Most of the nodules are in the granulation tissue stage.

The pathological diagnosis is: Periarteritis chronica nodosa; atrophica renis ex periarteriitide. Uremia.

In this case also the pains in the extremities, without any joint involvement dominated the clinical picture. An attack diagnosed acute nephritis followed two months after the onset of the disease. Then came an attack of orchitis, severe headaches and marked visual disturbance. The changes in the fundus are of great importance because their occurrence enables the ophthalmologist acquainted with periarteritis nodosa to make the diagnosis.

The hematuria and terminal uremia are quite characteristic of this disease. The hemorrhages in the central nervous system are unusual and have been seen in only nine cases to date. As in this case the diagnosis of acute nephritis is often made in the early stage because of hematuria.

**CASE 5. Clinical History:** The patient, Joseph S., entered the hospital on August 11, 1922. There was nothing of importance in the family history. The patient was never sick until 1918, when he became suddenly ill with a *high fever, severe icterus and "acute nephritis."* He was then in a hospital for six weeks and gradually recovered and returned to his work.

The present illness began in May, 1922 (four years later) with cramp-like pains in the epigastrium. These pains were so intense that the patient was forced to bed. He had a feeling of pressure in the stomach region, which was intensified by the taking of food. It was relieved by hot applications. There was no vomiting. Appetite was poor. The patient was constipated. Several weeks' treatment by a family physician was without effect and the patient entered the clinic on August 11, 1922.

The patient was much emaciated, the musculature atrophic. The skin was brown and pigmented. The mouth mucosa was not pigmented. The pupils reacted normally to light and accommodation. The veins of the neck were somewhat dilated.

A slight asymmetry of the thorax was present. The lungs were normal. The heart was slightly enlarged to the right; the heart sounds were normal. The pulse was regular, 90 to 100 per minute. The blood pressure was 150.

The abdomen was retracted. The spleen was not palpable. The liver could be palpated below the costal margin and seemed to have an irregular border. The patient complained of intense pain in the epigastrium.

On August 15, the patient revealed a dullness at the base of both lungs with signs of bronchitis. The apex beat was two finger-breadths outside the mid-clavicular line. On August 24, a marked edema of the legs had developed, as well as a generalized anasarca. There was dyspnea, the pulse was 120. The bilateral hydrothorax was increased. There was a systolic murmur at the apex of the heart. *In spite of digitalis therapy* the patient's edema increased and the quan-

great thickness, which made spasm of the vessels impossible, explains the absence of angina pectoris in this case.

We have here a new clinical syndrome due to histologically healed periarteritis nodosa: renal insufficiency, cardiac insufficiency and hepar lobatum. The finding of hepar lobatum in this disease makes it necessary to examine the vascular changes in such livers more carefully, instead of assuming the syphilitic nature of this condition. Also extensive fibrosis or atrophy of other organs may be due to periarteritis nodosa.

### THE IMPORTANT SYMPTOMS OBSERVED IN THE FIVE CASES HEREIN REPORTED

Accelerated regular pulse .....	5
Edema of the legs .....	5
Septic type of temperature .....	4
Pain in the extremities, polyneuritis (wrist-drop in two cases) .....	4
Hematuria .....	4
Cardiac insufficiency .....	3
Melena .....	3
Cerebral symptoms .....	2
Onset with acute angina.....	2
Abdominal pain .....	2
Changes in the fundus oculi .....	1

### SUMMARY

1. Periarteritis nodosa is a specific infectious disease probably caused by a filterable virus, with an elective affinity for the arteries of the body. The organs most commonly involved are the kidneys, heart, liver, muscles, peripheral nerves and gastro-intestinal tract. Any organ, or all may be affected.

2. The chief symptoms are a septic temperature, polyneuritis and polymyositis, hematuria or nephritis, abdominal cramp-like pains, progressive emaciation. The great variability of the symptoms, pointing to involvement of various organs, and the tendency toward acute exacerbations are suggestive of periarteritis nodosa.

3. The pathological changes in the arteries may be divided into four stages: (1) alterative-degenerative, (2) acute inflammatory, (3) granulation tissue, (4) histologically healed end-stage or scar tissue stage.

4. We have described the histologically healed end-stage of periarteritis nodosa. A patient with a single severe illness consisting of

the branches of the renal artery are thickened, some have nodules. Many are totally obliterated. Some of the nodules are spindle-shaped and surround the entire circumference, others are smaller and involve only part of the circumference. In places linear thickenings appear in the wall.

The mesenteric arteries are rigid and gaping with scattered small nodules in the wall. In the testis are several large infarcts. The gastric arteries are thickened and rigid, some show no visible lumen.

The histological examination of over fifty blocks of tissue from various organs revealed the fact that we are dealing here with the *histologically healed end-stage* or *scar tissue stage* of a *generalized periarteritis nodosa*, a *periarteritis obsoleta nodosa*. Nowhere is there evidence of acute inflammation. In practically all the cases hitherto described various stages of the inflammatory disease have been found, due to the acute exacerbations so common in this disease.

The characteristic changes found in this case are:

(1) Intima proliferation with new formation of elastic fibrils, leading to stenosis or even complete occlusion.

(2) Extensive destruction of the media including the elastica interna, or of the entire vessel wall, with aneurysm formation and thrombosis. The thrombosis is followed by complete organization, with here and there deposition of hemosiderin.

(3) A periarterial healed granulation tissue mantle consisting of dense fibrous connective tissue containing capillaries and hemosiderin deposits.

(4) Extensive destruction with even aneurysm formation in arteries with high grade intima proliferation.

(5) Healed infarct scars in most organs.

(6) High grade stenosis of both coronary arteries.

The consequence of the *periarteritis obsoleta nodosa* in our case was the development of: contracted kidneys, *hepar lobatum*, high grade coronary stenosis, infarction of the testicle, myomalacia scars, etc.

Death resulted from myocardial and renal insufficiency. Of great interest is the fact that the patient had no symptoms of angina pectoris, although both coronary arteries were reduced to one-fourth or less of their normal caliber. This finding shows us that a reduction of the blood supply to the myocardium need not cause angina pectoris. Perhaps the rigidity of the arteries due to their

have seen changes in the thyroid, adrenal, pancreas, ovary, and testis due to periarteritis nodosa.

9. How often periarteritis nodosa of a single organ or of numerous organs, comes to a complete standstill is difficult to say. We are inclined to consider complete histological healing, as in one of our cases, a rare occurrence. For in practically all the cases till now described acute as well as chronic changes have been present.

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icterus, high fever and acute nephritis died four years later of renal and cardiac insufficiency. The postmortem findings revealed a histologically healed end-stage of periarteritis nodosa affecting all the organs of the body except the central nervous system. The contracted kidneys, hepar lobatum, myomalacia scars, pancreatic and adrenal atrophy, and coronary stenosis were all due to this disease.

5. A new clinical syndrome characterized by cardiac insufficiency which failed to react to digitalis, renal insufficiency with low specific gravity of the urine and reduced chlorides, progressive emaciation, abdominal pain and hepar lobatum is described. Especially important is the fact that the patient lived four years after his single acute attack, and that the patient was entirely free from temperature during his fatal illness. The absence of temperature indicates histological healing of the disease.

6. Periarteritis nodosa is of interest to the surgeon because it can produce the symptoms of an acute cholecystitis with severe changes in the gall bladder, internal hemorrhage due to rupture of an aneurysm (kidney, liver, pancreas, brain, gastro-intestinal tract, lungs), or gangrene of the intestine with peritonitis. The great frequency of polyneuritis as the first and predominating symptom should interest the neurologist. In four of our five cases this was a prominent symptom. And the ophthalmologist who becomes acquainted with the disease may enable us to make the correct diagnosis *in vivo* by finding nodules or localized thickenings on the retinal arteries.

7. The diagnosis of periarteritis nodosa is very difficult, and it is only by keeping in mind the cardinal symptoms which we have described that the internist will be able to recognize the disease, after having ruled out other possibilities. In a few cases the diagnosis has been made by finding nodules in the skin with the characteristic histological changes in the blood vessels.

8. I wish to call attention to the fact that there is a microscopic form of periarteritis nodosa which can be recognized only by a careful study of tissues, especially with elastica stains of the blood vessels. Pathologists should examine carefully the arteries in atrophic organs or those with extensive fibrosis not to overlook changes such as we have described for the end-stage of periarteritis nodosa. It is possible that some cases of insufficiency of one or more glands of internal secretion may be due to atrophy caused by this disease. We

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## DESCRIPTION OF PLATES

### PLATE 89

- FIG. 1. (Case 5.) Healed periarteritis nodosa. A branch of the mesenteric artery with marked intimal proliferation with new formation of elastic fibrils. At this level the internal elastic layer is intact. Serial sections showed areas of wall destruction from which the intimal proliferation progressed.  $\times 150$ .
- FIG. 2. (Case 5.) Left coronary artery. This vessel presents an extensive destruction of about two-thirds of the circumference of the artery. The lumen has been greatly reduced in size by organized thrombi and intimal proliferation. There is also the characteristic periarterial healed granulation tissue mantle. Elastic tissue stain.  $\times 100$ .
- FIG. 3. (Case 5.) Pancreatic artery. Here the high-grade intimal proliferation predominates. At the other levels characteristic wall changes were found. Other arteries with complete obstruction by organized thrombi or intimal proliferation were found.  $\times 150$ .
- FIG. 4. (Case 5.) A bronchial artery at the lung hilum. This vessel presents remarkable changes due to wall destruction with aneurysm formations, high-grade intimal proliferation and final central thrombosis with organization and recanalization. Elastic tissue stain.  $\times 200$ .

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PLATE 90

- FIG. 5. (Case 5.) Para-esophageal artery, showing extensive destruction of the wall with aneurysm formation and complete organization. There is also stenosis of the lumen due to thrombosis with organization, and intimal proliferation. Elastic tissue stain.  $\times 150$ .
- FIG. 6. (Case 5.) Hepatic artery in healed periarteritis nodosa. Note the almost complete destruction of the artery, with organized thrombosis of the lumen, and a very thick periarterial vascularized healed granulation tissue. The acute changes must have been very extensive.  $\times 100$ .
- FIG. 7. (Case 5.) Liver in healed periarteritis nodosa. Note the large area of liver cell destruction, with beginning regeneration from some of the bile ducts. The branches of the hepatic artery show very marked changes, with total occlusion of many. The resulting infarction with organization produced deep scars with wrinkling of the liver capsule. The gross appearance resembled that seen in *hepar lobatum syphiliticum*. Hematoxylin and eosin.  $\times 75$ .
- FIG. 8. (Case 5.) Older infarcts of adrenal due to periarteritis nodosa. Only a small zone of cells at the periphery appears normal.  $\times 150$ .



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Arkin



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Periarteritis Nodosa

PLATE 91

- FIG. 9. (Case 1.) Acute periarteritis nodosa of the liver. This is the very early acute stage with hyaline necrosis and fibrinous exudation. There is some edema and cellular infiltration.  $\times 200$ .
- FIG. 10. (Case 1.) Acute periarteritis nodosa of the brain. This small arteriole reveals only a periarteriolar hemorrhage without any cellular infiltration. In other sections similar hemorrhages with wall changes were found.  $\times 300$ .
- FIG. 11. (Case 1.) Acute periarteritis nodosa of the sciatic nerve. This small artery reveals a very early acute stage, with fibrinous exudation and slight cellular infiltration. The process is in the media and subintimal connective tissue. The lumen has become V-shaped as a result of the exudation with elevation of the endothelium. This finding explains the eccentric lumen so often found in the arteries of the healed case.  $\times 200$ .
- FIG. 12. (Case 1.) Acute periarteritis nodosa of the kidney. This section shows an aneurysm formation, with thrombosis and early organization. The acute inflammation has subsided. Hematoxylin and eosin.  $\times 50$ .



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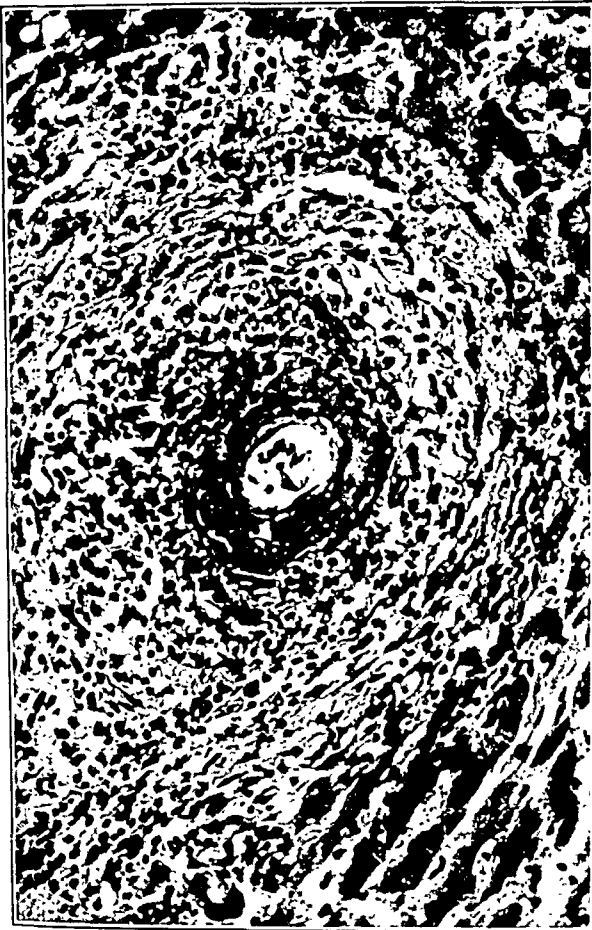
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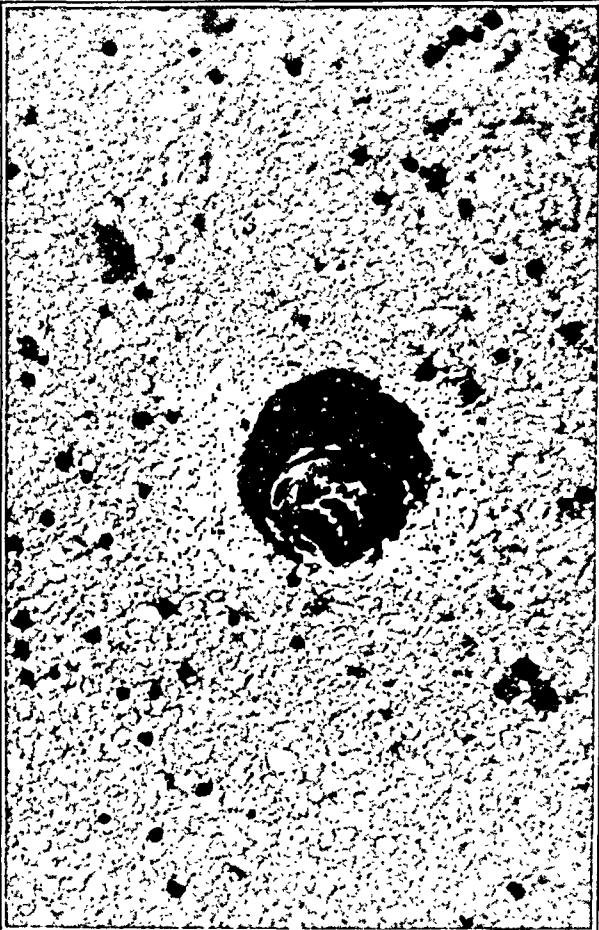
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Periarteritis Nodosa





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Arkin

Periarteritis Nodosa

By February 14, 1928, the small bloody tumor had assumed larger proportions. It was still freely movable, sharply outlined, and hard to the touch. There was no inguinal lymphadenopathy. A clinical diagnosis of *hemangioma* was made and the mass removed by Dr. C. F. Kivlin at the Troy Hospital. It was about 4 cm. in diameter, encapsulated, and contained many hemorrhagic areas as well as foci of pale, cellular appearing tissue. The pathological report was *sympathicoblastoma*, made by one of us (V. C. J.).

In August 22, 1928, the child was again operated on by Dr. Kivlin, for a recurrence of the tumor at the same site in the thigh beneath the scar. Physical examination of the rest of the body was negative.

*Gross Description of the Recurrent Tumor:* The specimen consisted of an ovoid mass, 8 by 3 by 3 cm., and a smaller one of about the size of a cherry. The larger growth lay subcutaneously but was visible externally as a red prominence. On cross-section, they were all hemorrhagic and spongy with very cellular, granular areas, grayish to opaque yellow in color (Fig. 1). They appeared entirely encapsulated and showed no demonstrable relationship to any vessel or nerve.

*Microscopic Findings:* One-half of the gross specimen was fixed in Zenker's solution and the other in 10 per cent neutral formalin. Paraffin sections were stained with hematoxylin and eosin, Van Gieson's picric-acid fuchsin, Mallory's phosphotungstic acid hematoxylin, Foot and Mènard's silver stain and Laidlaw's silver stain. Also, blocks were treated by Levaditi's method for myelinated nerve fibers.

All the sections are very cellular, with large and small hemorrhages scattered throughout. Thin strands of connective tissue group the tumor cells into variously sized alveoli. The tumor cells are round and about the size of small lymphocytes. They consist almost entirely of a hyperchromatic nucleus with a very narrow rim of faintly staining cytoplasm, scarcely discernible in many of the cells. Intimately connected with these tumor cells are numerous fine fibrillae, which stain faintly blue with hematoxylin and eosin, yellowish brown with the Van Gieson stain, dull blue with Mallory's phosphotungstic acid hematoxylin stain, and colorless with the different silver stains (Fig. 2). In other words, these fibrillae do not give the characteristic tinctorial reactions for connective tissue or neuroglia. Intermixed with these smaller tumor cells with dense hyperchromatic nuclei, are numerous slightly larger cells with a larger and less dense nucleus, surrounded by a larger amount of still faintly staining cytoplasm. These resemble the more differentiated

# PRIMARY SYMPATHICOBLASTOMA OF THE SKIN OF THE THIGH \*

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## INTRODUCTION

Since Wright<sup>1</sup> in 1910 first drew the attention of the medical world to the sympathetic neuroblastomas and reclassified the so-called round-celled sarcomas of the suprarenal medulla reported previously, many more tumors of this type have been recorded. At that time he stated that these growths could not be very rare as he himself observed five cases within one year. Boyd<sup>2</sup> encountered his three cases within a few months of one another. The three cases of Kwartin and Twiss<sup>3</sup> occurred within eighteen months. Yet in 1915, in an analysis of 2000 cases of malignant neoplasms in the young, Warthin<sup>4</sup> made no mention of the neuroblastoma. Saphir's case<sup>5</sup> was the only one among 3950 autopsies. The records of the pathological laboratory of the Albany Hospital for the last thirty years do not reveal a case of sympathicoblastoma. So far as we have been able to determine, a sympathicoblastoma of the skin of the thigh, primary or metastatic, has not yet been reported. For this reason the following case is of especial interest. We are indebted to Dr. C. F. Kivlin of Troy, New York, and Dr. A. E. Houle of Cohoes, New York, for the use of the clinical records of the patient.

## CASE REPORT

*Clinical History:* B. H., white, male, 9 months old. A paternal aunt died of carcinoma of the rectum, a maternal grandmother of cancer, the organ affected not being known.

His past history was quite uneventful. He was breast-fed. He slept, nursed, and digested his food well. The bowels were regular. He was normally developed for his age and well nourished, but the skin and mucous membranes were somewhat pale. Examination of the chest and abdomen revealed nothing abnormal.

On November 8, 1927, a small extravasation of blood appeared spontaneously on the anterior aspect of the left thigh. This area was about the size of a cherry, freely movable, painless to the touch, and evidently caused no pain, as the infant seldom, if ever cried.

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Two of Harbitz's cases<sup>13</sup> were in the sacral region. Alezais and Imbert<sup>14</sup> found their tumor connected with the glandula coccygea. In the cases of Wright,<sup>1</sup> Anderson and Shennan,<sup>15</sup> and Nieden,<sup>16</sup> the growths were situated in the thoracic cavity. In Martius' case,<sup>17</sup> the tumor originated in the cervical sympathetic and extended down into the thoracic cavity, completely surrounding the superior vena cava and the vena azygos. Capaldi's second case<sup>18</sup> is very similar in that the sympathicoblastoma of the cervical sympathetic extended down into the thoracic cavity and also invaded the spinal canal from the medulla oblongata to the eighth dorsal vertebra. His first case is very unique in that there were three primary tumors — one in the left inferior cervical sympathetic ganglion, another in the right inferior cervical ganglion, and the third in the retro-adrenal sympathetics. All three of these tumors invaded the spinal canal by direct extension. In Dunn's case,<sup>19</sup> the tumor was located over the right temple. Ritter<sup>20</sup> reported two case of neuroblastoma of the jejunum. MacNaughton-Jones and Turnbull<sup>21</sup> described a large ganglioneuroma (partly neuroblastomatous) of the mesentery. In Cushing and Wolbach's case,<sup>22</sup> the tumor was situated in the mid-scapular, right paravertebral region. The cases of Anitschkow, Capaldi, and Cushing and Wolbach are interesting in that the growths penetrated into the extradural spaces of the spinal canal through the intervertebral foramina, producing hour-glass tumors. Symmers<sup>23</sup> described a recurrent neuroblastoma of the scapular region although he mentions that, since the patient was lost sight of, its derivation from the adrenal capsule cannot be disclaimed. In the case here reported, the tumor was located in the skin of the thigh.

In addition to its origin from the sympathogonia, a neuroblastoma can theoretically arise as part of a teratomatous or teratoid process and thus be located anywhere along or near the midline where toti- or multipotential cells are liable to become misplaced. In one of Harbitz' cases, the finding of embryonic cartilage among the tumor cells of a neuroblastoma in the sacral region indicates that such an occurrence may have taken place there. In Suzuki's case<sup>24</sup> of a sympathetic adrenal tumor, fat cells of the signet ring type were also present. An overgrowth of the neuroblastoma cells or of any neoplastic tissue can efface most or all of the other identifying structures of the teratoma.

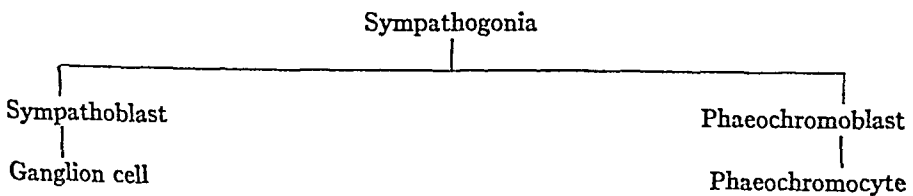
sympathoblast in contrast to the previously described sympathogonia or *bildungszellen*. As stained by Laidlaw's method, the nuclei of these tumor cells are all silver-positive, which is in agreement with Laidlaw<sup>6</sup> who stated that ectodermic cells retain the silver stain. There is no rosette formation but in places a tendency to sheaf-like arrangement. No ganglion cells are present anywhere. Collagen and reticulum are found only in the connective tissue septa of the tumor and in the walls of blood vessels.

The tumor cells have invaded the connective tissue capsule and infiltrated the surrounding fatty tissue. Even the dense fibrous layer just beneath the skin has become sparsely dotted with tumor cells. However, the various sections show that the neoplasm had been removed with a wide margin.

Sections were submitted to Dr. S. B. Wolbach of the Harvard Medical School, who concurred in the diagnosis of sympathicoblastoma.

#### DISCUSSION

When it is considered that the primitive nerve cells migrate from the neural crest to different parts of the developing embryo to form the future sympathetic system, any dysontogenetic factor may cause these primitive cells to become arrested anywhere in the body, later to develop into the various tumors derivative of the sympathogonia. The embryological development of the sympathogonia may be represented in the following schema (after Poll):



As regards the right branch of the schema, Rabin<sup>7</sup> has very recently made an excellent review of the benign phaeochromocytomas of the suprarenal medulla.

Sympathetic neuroblastomas have been observed elsewhere in the body than in the suprarenal glands. Landau,<sup>8</sup> Anitschkow,<sup>9</sup> Boyd,<sup>2</sup> and Wollstein<sup>10</sup> reported these growths as originating in the retroperitoneal sympathetics. Pick<sup>11</sup> and Lemeland and Durante<sup>12</sup> observed a "sympathoma embryonale" of the uterus.

Hagenbach lend support to our belief that a primary origin of the sympathicoblastoma in the skin of the thigh is not improbable. Our patient at the present writing is a vigorous child, almost two years after the removal of the recurrent growth, apparently normal in every way.

### SUMMARY

A case of sympathicoblastoma, primary in the skin or subcutaneum of the thigh of a nine-months-old infant, is here reported. There was a recurrence in the same location within six months. So far as we have been able to determine, this is the only instance of a sympathicoblastoma, occurring in such an unusual location. At the present writing, almost two years after the removal of the recurrent tumor, the child is vigorous, robust, and apparently normal in every respect.

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That neuroblastomas of the sympathetic may sometimes occur in the adult is shown by the recent papers of Meltzer<sup>25</sup> and of Blumensaat,<sup>26</sup> who collected four cases in addition to one of his own. The above-mentioned cases of Symmers, Ritter, and Leme-land and Durante were in adults. However, in general, the malignancy varies inversely with the age of the patient; that is, sympathicoblastomas are more common in the young and ganglioneuromas more common in adults. According to Pick and Bielschowsky,<sup>27</sup> we have the immature neuroma (neuroblastoma) and the mature neuroma (ganglioneuroma) depending on the varying degree of differentiation of the embryonal neurocytes.

Thus, microscopic findings have shown that all gradations and admixtures exist from the highly malignant tumor consisting mostly of the sympathetic *bildungszellen* type to the very benign tumor consisting wholly of ganglion cells and nerve fibers with or without the sheath of Schwann cells. Wright,<sup>1</sup> Harbitz,<sup>13</sup> Dunn,<sup>28</sup> Lehman,<sup>29</sup> Wolbach and Morse,<sup>30</sup> Saphir,<sup>5</sup> Matzdorff,<sup>31</sup> and others have described cases where the tumor cells were of the sympathoblast type, intermingled with the characteristically staining fibrillae with or without Kuester's rosettes. In the cases of Anitschkow,<sup>9</sup> Monro and Dunn,<sup>32</sup> Dunn,<sup>19</sup> Bülbring,<sup>33</sup> Wollstein,<sup>10</sup> and others, neuroblastomatous and ganglioneuromatous areas were intermingled; or the two parts may be distinct but connected together as in the case of Martius.<sup>17</sup> In the so-called malignant ganglioneuromas, it is the neuroblastomatous portions that gives rise to metastases. All three elements — neuroblastomatous, ganglioneuromatous and paraganglioneuromatous — were represented in the tumors reported by Hedinger,<sup>34</sup> Suzuki,<sup>24</sup> Wahl,<sup>35</sup> and Glomset.<sup>36</sup> There are already on record numerous examples of ganglioneuroma distributed throughout the body but very rarely intracerebrally. Some of the unusual cases are those of Knauss<sup>37</sup> and of Kredel and Beneke,<sup>38</sup> where the subcutaneous nodules of ganglioneuroma totalled over 60 and 160 respectively. Hagenbach's paper<sup>39</sup> is of special interest in that the ganglioneuroma in his case occurred in the region of the knee joint. It is to be noted here that the sympathicoblastoma in our case was located under the skin of the anterior aspect of the thigh. Although a malignant sympathetic tumor has not yet been observed in this location, the more highly differentiated tumor nodules observed subcutaneously in the cases of Knauss, Kredel and Beneke, and

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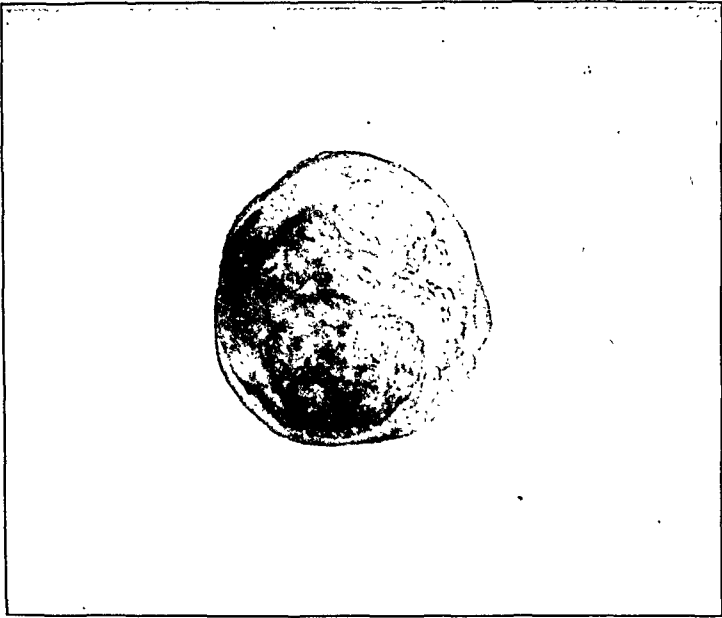
## DESCRIPTION OF PLATE

### PLATE 92

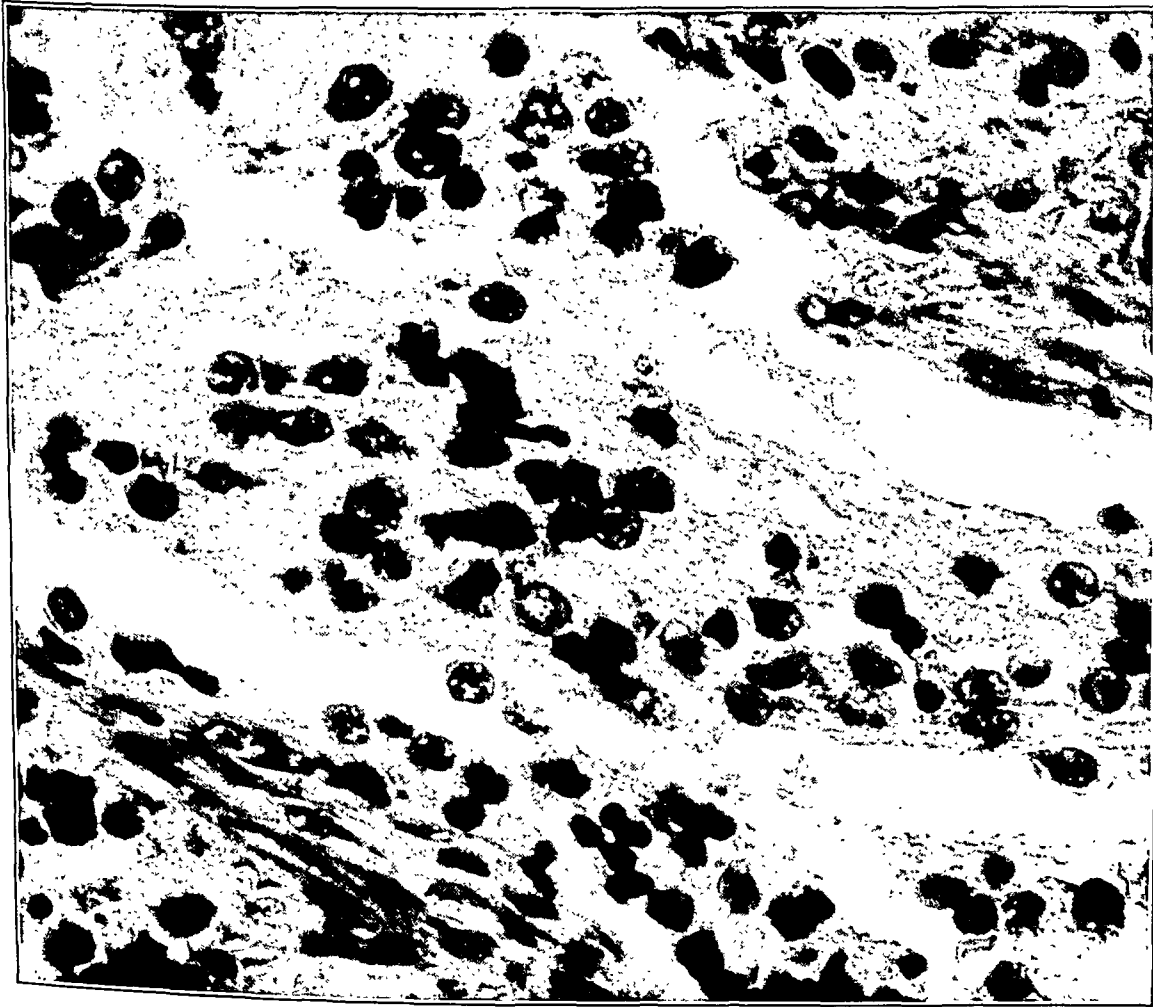
- FIG. 1. Photograph of a cross-section of the recurrent tumor of the skin of the thigh (after formalin fixation). The large dark area is due to marked congestion and hemorrhage. Necrotic foci in the lighter right quadrant.  $\times 1.4$ .
- FIG. 2. Photomicrograph showing the admixture of tumor cells of the sympathogonia and sympathoblast types and the fine fibrillary intercellular substance. Phosphotungstic acid hematoxylin.  $\times 778$ .

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I



2



*The web* sheathing the individual nerve fiber is revealed only by silver and only by silver used in a particular way. It has been seen by few. Studnička,<sup>13</sup> Snessarew,<sup>14</sup> and Ranke<sup>15</sup> note in passing that their respective silver techniques reveal "a network in the Schwann sheath and in the capsules of the ganglion cells." However, Plenk,<sup>16</sup> (1927, page 380), of the Histological Institute of Vienna, was really the first to describe and illustrate this delicate network which dips in at Ranvier's nodes and forms a closely fitting sheath around each nerve fiber. In the summer of 1928, Herr Plenk had the kindness to show me his preparations and to look at mine. I believe that it is fair to say that we agreed that the technique described in this paper gives the clearer view of the web. Plenk's work is a mine of information about argyrophil webs in all parts of the body and there is a full bibliography, from which the references in this paragraph were taken.

Here and there, the longitudinal fibers give off branches to the web and, at points where the nerve fibers have been torn apart, delicate filaments may be seen to pass from one web to another; these observations were made first by Nageotte.<sup>9</sup> While we have dealt with them separately for the purpose of description, longitudinal fibers and web undoubtedly form a whole and are to be regarded as the ultimate distribution of fibrous connective tissue around the individual nerve fiber.

We have found the same construction of the endoneurium in man, and in all of the laboratory animals examined, cat, dog, rabbit, rat, monkey and guinea pig.

### THE DORSAL ROOT GANGLION

From the fibrous capsule of the ganglion, septa of fibrous connective tissue extend inward, forming sheaths around each nerve fiber and around each ganglion cell. All this is demonstrable easily by the usual collagen stains; but, at this point, silver takes up the tale and reveals around each ganglion cell a closely plaited web of argyrophil fibers, as shown in Fig. 2. Here and there on the web are coarse, parallel fibers, representing the longitudinal fibers of the endoneurium.

In these argyrophil webs, there seems to be only one orifice, the point of exit of the axis cylinder. Fortunate sections show the argyrophil fibers woven neatly and smoothly around this orifice and

# SILVER STAINING OF THE ENDONEURIAL FIBERS OF THE CEREBROSPINAL NERVES \*

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The point of departure of any study of the connective tissue sheaths of the peripheral nerve must still be the work of Ranvier,<sup>1,2,3</sup> and of Key and Retzius.<sup>4,5</sup> Review of recent literature shows scarcely a line added to the descriptions given by these masters fifty years ago. The endoneurium is described by the one as strands of fibrous connective tissue, by the other as a sheath, lying in immediate contact with the individual nerve fiber. Obviously, the endoneurium consists of cells as well as fibers: we shall consider the fibers only.

The fibers of the endoneurium are revealed best by the silver methods that have been devised for the study of collagen; in the writer's opinion, the best of them is his modification<sup>6,7</sup> of Hortege's technique described in a former number of the Journal. We shall proceed to apply this method to the endoneurial fibers of a cerebrospinal nerve.

## THE DISTAL NERVE

As shown in Fig. 1, the endoneurial fibers of a distal nerve are arranged in two distinct patterns. There are longitudinal collagen fibers, and there is a delicate web around each nerve fiber.

The longitudinal fibers have been described by many authors. They constitute the *Fibrillenscheide* of Key and Retzius (1873,<sup>4</sup> page 354; 1876,<sup>5</sup> page 101), and the intrafascicular connective tissue of Ranvier (1875,<sup>1</sup> page 764; 1889,<sup>2</sup> page 585). Running between and over the nerve fibers, these longitudinal collagen fibers form a coarse network of meshes elongated in the direction of the nerve (Nageotte<sup>8,9</sup>).

The longitudinal fibers may be demonstrated fairly well by any good collagen stain but, like all fibers of the connective tissue group, they are brought out more effectively by silver. Stained with silver, they were described and illustrated by Ramón y Cajal (1909,<sup>10</sup> page 264; 1913,<sup>11</sup> page 76; 1928,<sup>12</sup> page 63).

\* Received for publication April 7, 1930.

be said that the peripheral nerve never succeeds wholly in getting outside of the pia mater. In cross-sections of such trunks as the sciatic and the tibial, these layers of the pia around the nerve bundle are recognized readily; even in the finest branches, the outer layer of the pia, the perineurium, continues as Henle's sheath.

### THE PIAL RING

The root slants upward and inward between the two layers of the intima piae and enters the cord through a hole in the inner layer. The margin of this hole is reinforced by a heavy ring of fibers from the intima piae. At the entrance of the relatively large sensory roots, the pial ring is strengthened further by fibrous partitions, dividing it into several smaller rings. Here and there, a small bundle of nerve fibers leaves the main bundle of the root and enters the cord through a small aperture of its own.

The pial ring merits our attention, for it explains the endoneurium. In favorable sections it can be seen plainly that the longitudinal fibers of the endoneurium spring from the pial ring. What appear to be holes in the inner layer of the intima piae are the points where its fibers stream out through the root around the individual nerve fibers to become the longitudinal fibers of the peripheral endoneurium.

### THE INTRAMEDULLARY ENDONEURIUM

As shown in Fig. 3, the endoneurium accompanies the nerve fibers of the root for a short distance into the cord; but there is a striking difference in its arrangement outside and inside of the pial ring. Outside, in the root, the strong "longitudinal" fibers run in all directions, binding the nerve fibers together into a bundle. Inside, where the nerve fibers are embedded in the substance of the cord, there seems to be no need of such collective support. Here the binding fibers are reduced to a few delicate filaments that can be traced from one nerve fiber to another. Inside of the cord, support is given rather to the individual nerve fiber by winding strong argyrophil fibers around and around it to form a tubular sheath, very like the tubular sheaths wound around the glomeruli in the ganglia. These winding argyrophil fibers can be traced to the pial ring. The pial ring, then, formed by the inner layer of the intima piae, supplies both kinds of fibers. Facing outward, the pial ring

at this point the argyrophil web of the ganglion cell is continuous with the argyrophil web of the nerve fiber.

Over the ganglion cell, the web is stronger and bolder than on the distal nerve. We shall see it become still denser and more intricate as we follow the root upward to and into the cord.

### GLOMERULI

Immediately on leaving the ganglion cell, the axis cylinder describes a curious convolution known as the glomerulus. The argyrophil web clings closely to the nerve fiber and accompanies it in all of its twistings and turnings. In the glomerulus, heavy argyrophil fibers run around and around the nerve fiber, forming a tubular sheath which gives an impression of resistance and rigidity. The arrangement suggests the spiral wire reinforcement around a rubber garden hose. Counterstaining with azo carmin shows the winding axis cylinder inside of the argyrophil tube.

### THE SPINAL NERVE ROOTS

Fig. 3 shows a sensory root entering the cord. In the roots, both the longitudinal fibers and the web are heavier and more prominent than in the distal nerve. Here the plexus arrangement of the endoneurium is particularly evident, heavy argyrophil fibers crossing the nerve bundle in all directions. As in the distal nerve, at points where the nerve fibers have been torn apart, delicate web filaments are seen to pass from one nerve fiber to another, (Nageotte<sup>9</sup>).

### THE PIA

Silver brings out in striking contrast the two layers of the medullary pia described by Key and Retzius <sup>5</sup> (1875, page 143). As the root joins the cord, it is seen clearly that endoneurium and perineurium are merely peripheral extensions of these two layers of the pia mater. The outer layer of the pia consists of concentric laminae of dense collagen; it continues out over the root as the laminated perineurium.

The inner layer, the intima piae of Key and Retzius, is a loosely woven network of collagen and reticulum fibers that splits into two layers to surround the roots. The outer layer of this intima piae extends out over the root just beneath the perineurium. In fact, it may

*For Distal Nerves:*

1. Large nerves should be split into thin slices to ensure rapid penetration. Fix in Zenker from 3 to 5 hours, no longer. Wash in running water from 3 hours to overnight, as convenient.

2. Embed in paraffin.

3. Stick sections on the slide with Masson's gelatin glue; harden the gelatin in hot formol fumes overnight (*Am. J. Path.*, 1928, 4, 206; *ibid.*, 1929, 5, 245).

4. After removal of the paraffin, wash in running water for 5 minutes.

5. Mordant with the Mallory bleach:

(a) 1 per cent tincture of iodine, 3 minutes; rinse in tap water.

(b) 5 per cent hypo, 3 minutes; rinse in tap water.

(c)  $\frac{1}{4}$  per cent potassium permanganate, 5 minutes; rinse in tap water.

(d) 5 per cent oxalic acid, 5 minutes; wash well in running water for 10 minutes.

6. Distilled water; change 3 times within 5 or 10 minutes.

7. Río-Hortega's lithium silver augmented to 10 per cent at 55 to 58° C. for 5 minutes.

8. Quick rinse by pouring distilled water over both sides of the slide.

9. Formol, 1 per cent in tap water, 3 minutes.

10. Rinse with distilled water.

11. Yellow gold chlorid, 1 to 500, at room temperature, 10 minutes.

12. Rinse with distilled water.

13. Oxalic acid, 5 per cent, 10 minutes.

14. Rinse with distilled water.

15. Hypo, 5 per cent, 10 minutes; change as often as it becomes turbid.

16. Wash well in running water to remove the hypo.

Counterstain as desired and mount in balsam. The best counterstains are the reds, such as erythrosin, 1 per cent, or azo carmin,  $\frac{1}{2}$  per cent.

The silver solution and the gold solution may be used again and again. Filter the silver solution before use.

gives off the longitudinal fibers of the peripheral endoneurium; facing inward, it supplies tubular sheaths to the nerve fibers embedded in the cord.

These argyrophil tubes are seen especially well in thick sections, 20 to 25 microns. After penetrating the cord for a short distance, the bundle of tubes stops abruptly as if chopped off by a knife. The tip of each tube is conical, rounded off as neatly as if turned in a lathe. In many sections, a pale pink axis cylinder from the cord may be seen to enter each conical tip and run inside of the tube formed by the argyrophil web. Counterstaining with azo carmin brings out clearly the position of the axis cylinder inside of the argyrophil web. Motor and sensory roots present exactly the same structure.

### THE PIAL FUNNELS

At the points where blood vessels from the pia enter the cord, Key and Retzius (1876,<sup>5</sup> page 5 and Table I) describe funnel-shaped extensions of the intima piae (*Piatrichter*) sunk into the cord, forming a loose sheath around the vessel. In silvered sections, these funnels are seen clearly outlined in black. As stated by these authors, here and there a root may enter the cord through such a pial funnel. Most of the roots penetrate the cord accompanied only by the argyrophil endoneurium and the membrane of Schwann.

### THE MEMBRANE OF SCHWANN

Ranvier (1875,<sup>1</sup> page 1073; 1882,<sup>3</sup> page 1069; 1889,<sup>2</sup> page 805) stated that the membrane of Schwann accompanies the root fibers for a short distance into the marginal glia of the cord and he gives an illustration that is strikingly like Fig. 3. According to Nageotte, this observation has been forgotten. Inside of the cord, the membrane of Schwann and the argyrophil endoneurial sheath have exactly the same distribution.

### TECHNIQUE

We shall give here only a résumé of the technique as modified for the peripheral nerves, referring the reader to the paper in a former number of the Journal (1929) where the methods and the formulas were described in detail.

The universal dependence on formol fixation may be the chief reason why these details were not described long ago.

For distal nerves Zenker is the fixative of choice. For ganglia and roots, Zenker is one of the best fixatives but it is highly selective and somewhat erratic; it sensitizes the various components of the tissue in different degrees and demands some care in selecting the time in the fixative and the temperature and concentration of the silver bath. For ganglia and roots Zenker is a fixative for the expert; Bouin is the fixative for the routine worker, for with routine methods it gives uniform results.

### SUMMARY

The endoneurium consists of longitudinal fibers and a closely fitting argyrophil web. The distribution of the web is described together with the silver technique necessary for its demonstration.

This study was commenced and in great part completed in 1927 in the Laboratory of Neurocytology of the Presbyterian Hospital of New York City, at the instance of the Director, Wilder Penfield. The writer takes this occasion to thank both Professor Penfield and Professor William V. Cone for cordial assistance of every kind. That modern master of the histology of the peripheral nerves, Professor Nageotte, of the Collège de France, has been good enough to review and confirm these observations. The writer remains ever indebted to him for sound criticism and advice.

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*For Ganglia:*

1. Fix in Bouin's fluid from 1 to 3 days; pass directly to absolute alcohol.

2. Embed in paraffin.

Subsequent steps as for distal nerves except that, in Step 4, Bouin sections should be washed in running water for 20 minutes to remove the picric acid thoroughly; and, in Step 7, the temperature and concentration of the silver bath should be lower. Ten per cent lithium silver at room temperature for 10 minutes or 2 to 3 per cent silver at 40 or 45° C. for 5 minutes give cleaner lines and better detail than the higher temperatures that are necessary for distal nerves.

As noted in the former paper, after Bouin fixation this technique differentiates ectodermic from mesodermic cells. Correspondingly, while all mesodermic cells are invisible, the ectodermic ganglion cells are silver-positive. To obliterate the ganglion cells and secure a pure picture of the collagen framework, as in Fig. 2, we have found several methods effective. The most reliable of them is to repeat the Mallory bleach. After the first Mallory bleach, leave the sections overnight in distilled water, changing it several times. The next day, repeat the bleach and continue from Step 6 as usual.

Ganglia fixed in Zenker not more than 3 hours and stained with silver at 40° C. or under, show good webs and colorless ganglion cells.

*For Spinal Nerve Roots:*

Fix in Bouin and treat as ganglia. The cord is likely to stain red or black, giving poor contrast with the black web on the roots. Here the double Mallory bleach is useless. A paler ground is secured by a quick rinse with weak ammonia water after the silver bath. The ammonia rinse should not be too long or too strong or the web on the root will be decolorized also. We add 5 drops of ammonia to 100 cc. of distilled water and pour it over the slide for exactly 5 seconds by the watch; then rinse quickly with distilled water and proceed from Step 8 as usual. If the ammonia is to be used, stain the sections at 40 or 45° C. Sections stained at room temperature decolorize too easily.

*Zenker, Formol and Bouin Fixation:*

Formol fixation may be rejected at once. In formol-fixed sections, the web and the finer details of the endoneurium remain invisible.



## DESCRIPTION OF PLATES

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### PLATE 93

FIG. 1. Sciatic nerve of cat. Paraffin section. The author's silver technique. The endoneurial fibers (longitudinal fibers and web). The axis cylinders, Schwann cells and myelin sheaths (neurokeratin) are invisible.

FIG. 2. Dorsal root ganglion of cat. Paraffin section. Author's silver technique. A pure picture of the framework of the ganglion; all else invisible. In the upper part of the figure, the web forms fibrous capsules over several (invisible) ganglion cells; over many of the cells it has been cut away.

Center of figure, portion of a glomerulus inside of a fibrous capsule; below this, an entire glomerulus and two fibrous capsules with tops cut off.

Lower part of figure, the endoneurium of the root fibers, showing the longitudinal fibers and the web.

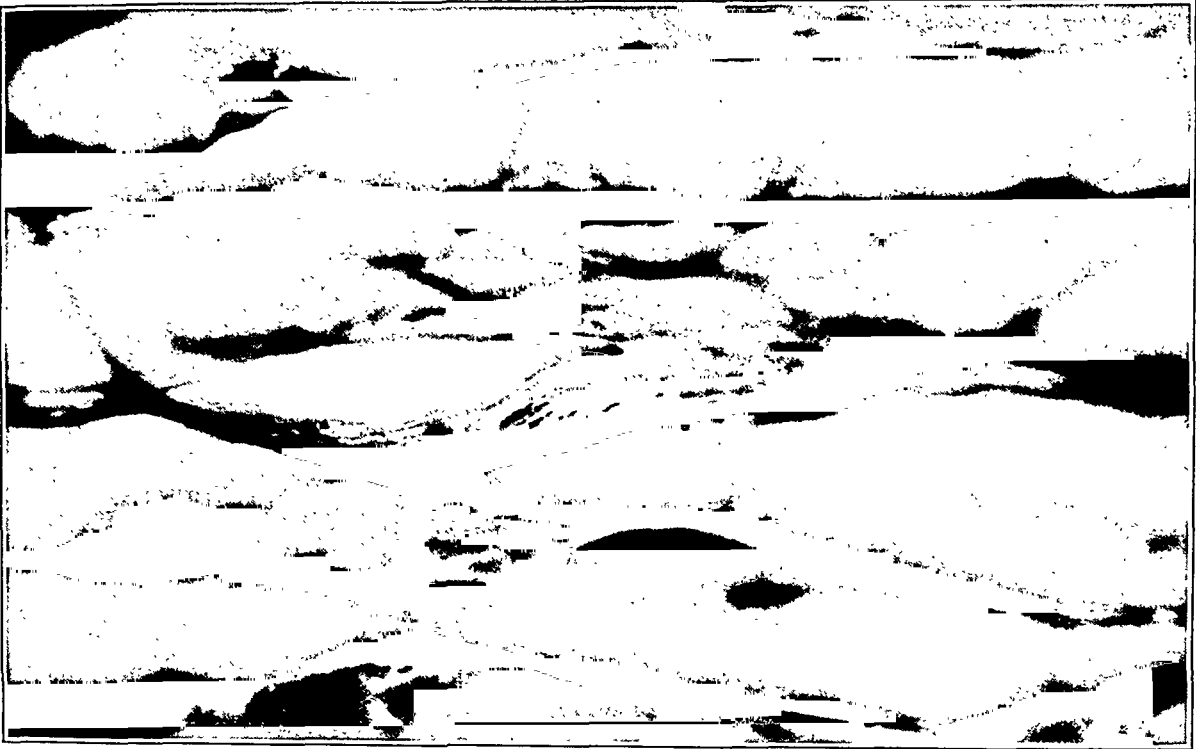
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PLATE 94

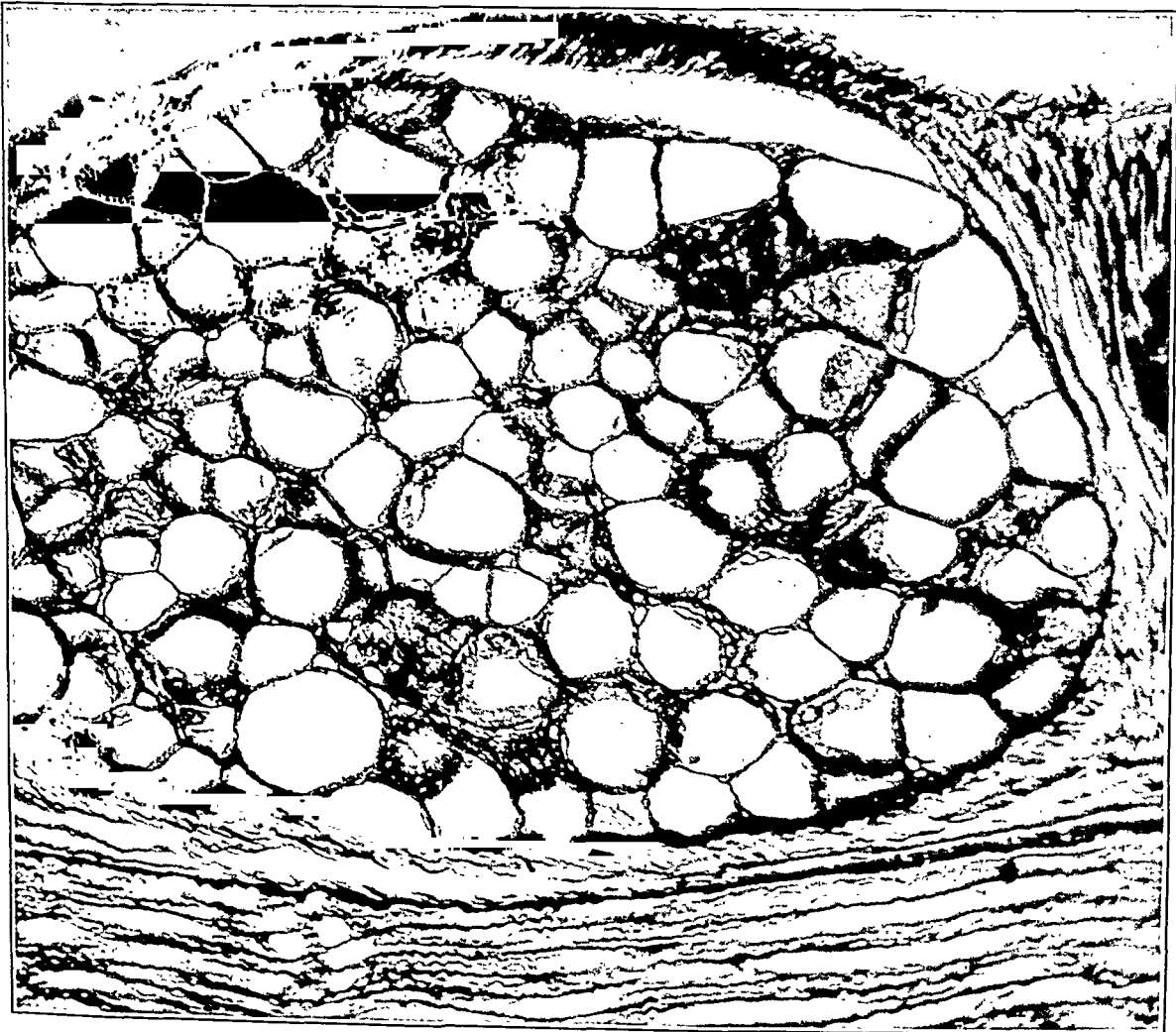
FIG. 3. Cross-section of cord of cat; entrance of sensory root. Paraffin section. Author's silver technique.

Upper left, the endoneurium of the root with prominent longitudinal fibers and web. Center, the pial ring, forming two loops. The longitudinal fibers of the root are continuous with those of the pial ring.

Within the pial ring, the intramedullary endoneurium, accompanying the nerve fiber for a short distance into the cord. Here the longitudinal fibers are few and inconspicuous. The intramedullary endoneurium is seen to consist chiefly of spiral or circular fibers given off by the pial ring: it ends abruptly in a conical tip from which the axis cylinder emerges.



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3. *Wash* rapidly in 60 per cent alcohol.\* The section should be carried through with a small angulated glass rod so as to allow all of it to be washed equally, without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.

4. *Reduce* by passing sections directly into 1 per cent formalin.

5. *Wash* in distilled water.

6. *Tone* by placing sections in gold chloride toning bath † 10 or 15 minutes until they become purple-gray in color.

7. *Fix* in 5 per cent hyposulphite of soda for  $\frac{1}{2}$  minute or more until sections are flexible.

8. *Wash* in water.

9. *Dehydrate* in dishes of graded alcohol followed by clearing in carbol-xytol-creosote.‡

10. *Mount* on slide in Canada balsam.

Best results as a rule are obtained by leaving tissues for 5 to 20 hours in the fixative which, for the sake of brevity, is called F. U. P. I. or fupi. But the staining capacity may be revived in an overfixed subject by placing sections in 4 per cent urea overnight and then passing them directly into the silver bath for an hour or less. If the subject has been fixed by preliminary carotid injection, it is better to try for oligodendroglia within 2 hours or less after the block has been placed in fixative.

To stain oligodendroglia in sections of the *spinal cord*, *optic nerve* and *retina*, place small fresh pieces in the above fixative to which has been added 4 gm. of chloral hydrate. Our best results were obtained by leaving fresh blocks of tissue from 2 to 4 days in this fixative. Cut sections and proceed as above.

Creditable staining of oligodendrocytes has been obtained from old formol material in the following manner: Blocks were cut and placed in 15 per cent ammonia water for 24 hours. They were then washed in running tap water overnight and placed in the F. U. P. I. fixative for a week. Sections were then cut and left in 4 per cent urea overnight and stained as before. This applies to brain tissue. We have had no marked success with old formalin-fixed spinal cords.

\* Commercial alcohol (95 per cent) usually contains some impurity. When diluted there appears a slight opalescence. This is prejudicial to the success of the staining. For this reason we have always used absolute alcohol in preparing the 60 per cent.

† Toning bath: Gold chloride (yellow) ..... 1 gm.  
Distilled water ..... 500 cc.

‡ Carbolic acid 10 cc., creosote 10 cc., xytol 80 cc.

# A FURTHER MODIFICATION OF DEL RÍO-HORTEGA'S METHOD OF STAINING OLIGODENDROGLIA \*

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This modification has been worked out with untiring enthusiasm in our laboratory by the senior technician, Mr. Edward Dockrill. In recognition of this fact it is proposed that the method be called Dockrill's Modification of the silver carbonate method for oligodendroglia.

The method is particularly reliable for staining the oligodendrocytes of the spinal cord, brain stem and cerebral white matter, where other methods are less often successful. It also stains the *sheath of Schwann cells* on the peripheral nerves selectively.

## FIXATION

Fresh tissue should be fixed in the following solution for 2 hours or up to 1 or 2 days.

Fixative (F. U. P. I.)	{ Formalin (40 per cent commercial) .....	20 cc.
	{ Urea .....	4 gm.
	{ Potassium iodide .....	6 gm.
	{ Water (doubly distilled) .....	80 cc.

Cut sections at about 15 microns on the freezing microtome and place in distilled water.

## STAINING METHOD †

1. *Wash* in two dishes of distilled water, the first containing 10 drops of ammonia.

2. *Stain* in undiluted silver carbonate ‡ from 1 minute to 1½ hours.

\* Received for publication April 28, 1930.

† The numbers correspond with those in Text-Figure 1.

‡ The solution is del Río-Hortega's undiluted ammoniacal silver carbonate made up carefully as follows:

Solution of silver nitrate (Merck) 10 per cent .....	5 cc.
Solution of sodium carbonate (pure) 5 per cent .....	20 cc.
Ammonium hydroxide (sufficient to dissolve precipitate).	

The ammonium hydroxide, as indicated above, should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. Finally, filter and place in a dark bottle, where it will keep for long periods.



## DESCRIPTION OF PLATE

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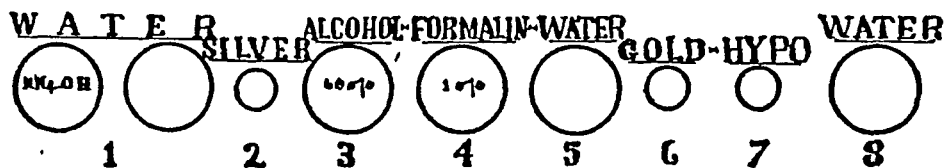
### PLATE 95

FIG. 1. Rows of oligodendrocytes in the cerebral white matter of a dog; normal.

FIG. 2. Higher magnification of oligodendrocytes from the white matter of the same animal.

## RESULTS

More complete staining of the oligodendrocytes in the white matter of the brain and spinal cord may be obtained by this modification of del Río-Hortega's method (Figs. 1 and 2), although the results in the gray matter are less delicate and satisfactory than by the original method of that author<sup>1</sup> or the modification for microglia and oligodendroglia by Penfield.<sup>1</sup>



TEXT-FIGURE 1

Order of staining procedure from left to right. Numbers correspond with the text.

The recently described method of del Río-Hortega,<sup>2</sup> which is a modification of Golgi's chrome silver method, occasionally gives results which for complete staining of the oligodendrocyte expansions are unequalled. But the results are so unequal and the staining so powdery as to make it so far of little use for routine work.

The method described here has been found particularly useful by Dr. Cone in staining the oligodendroglia of the retina, nerve head and optic nerves. Microglia is also stained with varying success by this method.

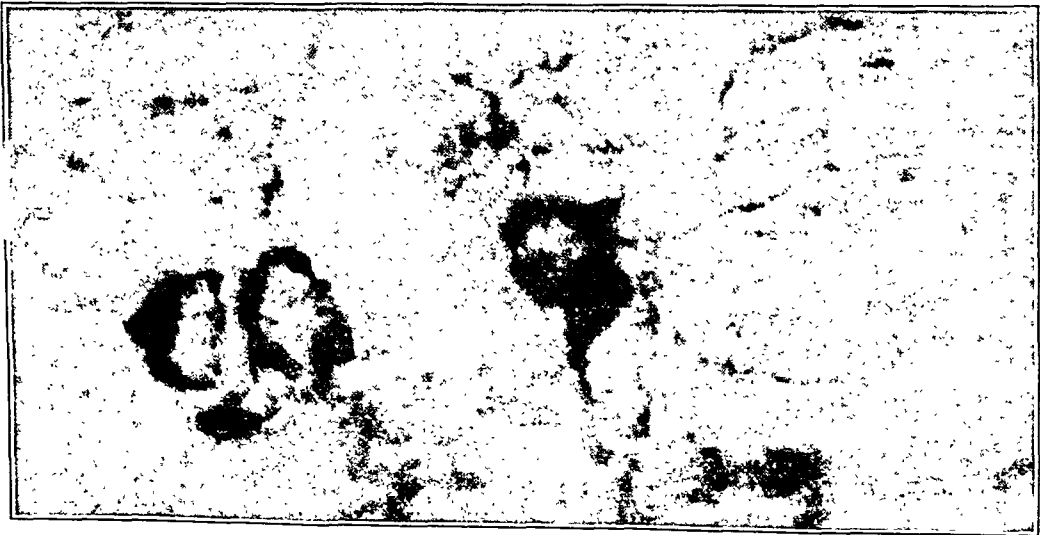
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sodium citrate (respectively 0.5 and 1 per cent), acidifying faintly with hydrochloric acid, barely bringing to the boiling point and neutralizing. This treatment does not injure tuberculin. As noted in the previous article, it is necessary to inject particulate matter for the success of this experiment, in order to localize the effects of the substance. Injecting clear, fluid tuberculin elicits only the general effects of this substance. Injection was made in exactly the same manner as in the first investigation. The effect of the injections was determined by biopsy two to eight days later, and by the final picture at autopsy.

All animals were subjected to the tuberculin skin test before the injections were made, and distinct differences were noted in the three groups of animals. All animals were infected with the human type of tubercle bacillus except in the case of three goats. Goats proved highly susceptible to bovine infection, the three animals injected succumbing in four to six weeks with widely disseminated tuberculosis, from a subcutaneous injection of 2 milligrams of bacilli (see protocols for Goats 1, 2 and 3 below).

All infected animals were skin sensitive in some degree to tuberculin. In the monkeys the zone of cutaneous reaction was wide in extent but extremely soft and pale. The red injection and the induration characteristic of the reaction in the guinea pig and man were absent. The goats gave strong reactions, with a redness and firm area of inflammatory edema surpassing that which we have seen in any other animal. The swine gave red zones of inflammatory induration of feeble intensity.

The monkeys (*Macacus rhesus*) averaged about five pounds in weight, the goats thirty pounds, and the pigs forty (at the outset). The results of the experiment are summarized in the following protocols.

## MONKEYS

### *Monkey 1. Tuberculous*

*Feb. 25, 1928:* 0.1 mg. of H 37 tubercle bacilli injected intraperitoneally.

*April 15, 1928:* Tuberculin test weakly positive.

*May 4, 1928:* Right kidney injected with approximately 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

## RESULTS FOLLOWING INTRARENAL ARTERIAL TUBERCULIN INJECTIONS IN NORMAL AND TUBERCULOUS MONKEYS, GOATS AND SWINE \*

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In a previous investigation<sup>1</sup> it was shown that injection of the specific protein of tuberculin into the renal artery of tuberculous swine resulted in acute inflammation involving glomeruli and interstitial tissue. The fact that similar injection into normal swine failed to cause an inflammatory reaction, indicated that the effect in the tuberculous animal was a true tuberculin reaction.

The experiments were not reported as suggesting a relationship between tuberculosis and spontaneous nephritis in man, but simply as an example of one possible effect, in a chronically infected and therefore hypersensitive animal, of the substance to which the animal is hypersensitive. The results seemed of possible significance for the general field of chronic infection and allergy.

The dosage used to produce the effects recorded, however, while insufficient to produce effects in normal control animals, was nevertheless much larger than any amount that could be liberated spontaneously from a focus of disease in any infected animal. It therefore seemed necessary before application could be made to spontaneous nephritis, to determine if much smaller amounts could produce lesions like those of naturally occurring disease. It seemed desirable, also, to determine if the condition noted for swine held for other animals.

Accordingly a study was made of the effect of smaller amounts of tuberculin protein in monkeys and goats. A number of swine were again studied. The purified tuberculin protein of Florence Seibert, prepared by ultrafiltration,<sup>2</sup> was used, and in amounts varying from 5 to 35 milligrams. The material for injection was secured in the form of a fine flocculent suspension by diluting the pure solution of tuberculin protein with an anticoagulating solution of salt and

\* Aided by a grant from the Medical Research Committee of the National Tuberculosis Association.

Received for publication April 17, 1930.

*July 17, 1928:* Monkey died. Autopsy: generalized abdominal lymph node tuberculosis. Right kidney: no changes of significance. Left kidney: normal.

*Monkey 4. Normal*

*May 15, 1928:* Tuberculin test negative.

*May 25, 1928:* Right kidney perfused with approximately 5 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*June 5, 1928:* Biopsy of right kidney. No changes of significance found. After the biopsy this monkey was transferred to another experiment. A second operation was not performed, as we were interested at the time only in the effect of tuberculin protein on the absolutely normal animal.

*Monkey 5. Normal*

*May 15, 1928:* Tuberculin test negative.

*June 4, 1928:* Right kidney perfused with 5 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*June 11, 1928:* Biopsy of right kidney. No changes of significance found. After the biopsy this monkey also was transferred to another experiment.

A number of determinations of blood chemistry were made on the monkeys of this series by Dr. Lucy Finner, but at no time were values outside of the normal range encountered.

GOATS

*Goats 1 and 2. Tuberculous*

*July 23, 1928:* 2 mg. of bovine type tubercle bacilli (B 1698) injected in left groin, and 2 mg. of human type bacilli (H 37) injected in right groin. In the succeeding weeks the lymph glands on the side of the bovine infection increased greatly in size, while those on the side infected with human bacilli increased only moderately. Neither of these goats lived long enough to be used for the experiment. Goat 1 died on Sept. 3, 1928, and Goat 2 on Sept. 7, 1928, each with very extensive pulmonary miliary tuberculosis.

*May 11, 1928:* Biopsy of right kidney. Specimen showed no changes of any significance.

*June 1, 1928:* Left kidney similarly injected.

*June 4, 1928:* Biopsy of left kidney. Specimen showed localized regions of degeneration from vascular injury, and a more diffuse involvement characterized by the presence of many dense hyaline casts. Many of these contained polymorphonuclear leucocytes. No other inflammatory changes were seen.

*July 12, 1928:* Monkey died. Autopsy: generalized abdominal lymphatic tuberculosis. Sections from the right kidney (injected May 4) showed no changes except a few small zones of lymphocytic infiltration. The left (injected June 1) was similar, except that, in addition, a few healed infarcts were present.

#### *Monkey 2. Tuberculous*

*Feb. 25, 1928:* 0.1 mg. of H 37 injected intraperitoneally.

*April 15, 1928:* Tuberculin test weakly positive.

*April 27, 1928:* Right kidney injected with approximately 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*May 4, 1928:* Biopsy of right kidney. The only changes from the normal were a slight proteinuria, and a few old scars infiltrated with lymphocytes, which presumably dated from an unknown, much older injury.

*May 21, 1928:* Left kidney similarly injected.

*May 29, 1928:* Biopsy of left kidney. Some cortical necrosis due to vascular injury was found, and casts were numerous throughout the section. Practically no inflammatory changes were seen.

*July 13, 1928:* Monkey died. Autopsy: generalized abdominal and lymph node tuberculosis. In the right kidney no changes of significance were seen. The left kidney was normal except for cortical regions of healed infarction.

#### *Monkey 3. Tuberculous*

*Feb. 25, 1928:* 0.1 mg. of H 37 injected intraperitoneally.

*April 15, 1928:* Tuberculin test weakly positive.

*May 14, 1928:* Right kidney perfused with approximately 5 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*May 23, 1928:* Biopsy of right kidney. No changes of significance found.



*Nov. 26, 1928:* Left kidney injected similarly.

*Nov. 28, 1928:* Biopsy of left kidney. Specimen showed no changes except marked proteinuria. No appreciable tubular degeneration, no casts, no inflammatory changes.

*Jan. 9, 1929:* Tuberculin test much weaker than on October 25.

*Jan. 19, 1929:* 4 mg. of H 37 injected in right groin.

*Mar. 1, 1929:* Tuberculin test strongly positive.

*Mar. 13, 1929:* Right kidney exposed for second injection, but found completely atrophied (complete infarction from vascular injury).

*July 15, 1929:* 1 mg. of bovine tubercle bacilli injected in right axilla.

*Aug. 29, 1929:* Left kidney exposed, biopsy specimen taken and 10 mg. of tuberculin protein injected into renal artery. The biopsy specimen resembled that seen on Nov. 28, 1928.

*Oct. 11, 1929:* Goat killed. Right kidney could not be found. Left kidney showed little change grossly. Microscopically a number of regions of periglomerular lymphocytic infiltration were seen, and a few small fibrous scars. Fusion of tuft and capsule was seen in a few glomeruli. Casts were present in many of the tubules. The lymph nodes regional to the points of injection were the seat of a mild fibrocaseous tuberculosis. There was no disseminated tuberculosis.

#### *Goat 5. Tuberculous*

*Sept. 10, 1928:* Tuberculin test negative.

*Sept. 20, 1928:* 3 mg. of H 37 injected in left groin.

*Oct. 25, 1928:* Tuberculin test strongly positive.

*Nov. 5, 1928:* Left kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*Nov. 7, 1928:* Biopsy of left kidney. Section revealed marked proteinuria, and no inflammatory changes.

*Dec. 3, 1928:* Right kidney injected similarly.

*Dec. 5, 1928:* Biopsy of right kidney. Section revealed a marked proteinuria and many dense hyaline casts in the tubules. Mitotic figures were numerous in the tubular epithelium. A few minute regions of periglomerular lymphocytic infiltration were seen, and minute collections of polymorphonuclear leucocytes were occasionally found in the glomerular capillaries.

*Goat 3. Tuberculous*

*July 23, 1928:* 2 mg. each of bovine type tubercle bacilli (B 1698) and human type bacilli (H 37) injected into opposite groins. In the succeeding weeks the glands on the side of the bovine infection increased greatly in size, while those on the side infected with human type bacilli increased only moderately.

*Sept. 1, 1928:* Tuberculin test strongly positive.

*Sept. 10, 1928:* Right kidney injected with a suspension of 10 mg. of coagulated tuberculin protein in salt-citrate solution.

*Sept. 12, 1928:* Biopsy of right kidney. Marked degeneration of the tubular epithelium with some interstitial infiltration with polymorphonuclear leucocytes was found. The glomeruli were unchanged except for the presence of a good many leucocytes. Many of the tubules contained hyaline casts.

*Sept. 17, 1928:* The left kidney was similarly perfused with a suspension containing 20 mg. of coagulated tuberculin protein. Marked shivering developed in the animal during the injection.

*Sept. 19, 1928:* Goat died. Autopsy revealed very extensive pulmonary miliary tuberculosis. Sections of the right kidney showed extensive interstitial lymphocytic infiltration, in place of the polymorphonuclear leucocytic reaction seen in this kidney on September 12. Much regeneration of the epithelium of this kidney was found. Many hyaline and leucocytic casts were present in the tubules. The glomeruli appeared unchanged. The left kidney showed changes similar to those seen in the right kidney September 12, except that the inflammatory features were less marked. Much degeneration of the tubular epithelium was seen.

*Goat 4. Tuberculous*

*Sept. 10, 1928:* Tuberculin test negative.

*Sept. 20, 1928:* 3 mg. of H 37 injected in left groin. In succeeding weeks marked enlargement of the regional inguinal glands developed.

*Oct. 25, 1928:* Tuberculin test strongly positive.

*Oct. 31, 1928:* Right kidney perfused with 10 mg. of coagulated tuberculin protein in salt-citrate solution. A mild general reaction with shivering occurred.

*Nov. 2, 1928:* Biopsy of right kidney. Specimen showed vascular injury and anemic necrosis. No inflammatory changes were present.

*Oct. 22, 1928:* Right kidney similarly injected.

*Oct. 24, 1928:* Biopsy of right kidney. Sections revealed no changes except proteinuria.

*Feb. 13, 1929:* Right kidney again injected, with 30 mg. of coagulated protein.

*Feb. 15, 1929:* Right kidney exposed for biopsy and found completely infarcted. Renal artery thrombosed. Kidney removed.

*Oct. 19, 1929:* Goat killed. Left kidney (injected Oct. 10, 1928) essentially normal.

#### *Goat 8. Normal*

*Sept. 10, 1928:* Tuberculin test negative.

*Oct. 17, 1928:* Right kidney injected with 10 mg. of coagulated tuberculin protein. Injection attended with unusual amount of trauma.

*Oct. 19, 1928:* Biopsy of right kidney. Sections for the most part quite normal. One infarcted region full of casts found; tubules in this region swollen and vacuolated; thrombosed artery not found in section.

*Nov. 19, 1928:* Left kidney injected in similar manner.

*Nov. 21, 1928:* Biopsy of left kidney. Section revealed no changes except proteinuria.

*Dec. 6, 1928:* Goat died, with ascites and bilateral hydrothorax. Both kidneys were found completely infarcted from thrombosis of the renal arteries.

#### SWINE

##### *Pig 1. Tuberculous*

*May 1, 1929:* Infected in groin with 2 mg. of human type tubercle bacilli.

*June 4, 1929:* Tuberculin test positive, moderate degree.

*June 10, 1929:* Right kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*June 12, 1929:* Biopsy of right kidney. Moderate changes were seen with hematoxylin and eosin stains. The tubular epithelium was swollen. Many hyaline and a few leucocytic casts were seen in the tubules. A few patches of interstitial lymphocytic infiltration

*Dec. 10, 1928:* Goat very sick.

*Dec. 11, 1928:* Goat died with generalized peritonitis. Sections showed both kidneys practically normal.

*Goat 6. Tuberculous*

*Sept. 10, 1928:* Tuberculin test negative.

*Sept. 20, 1928:* 3 mg. of H 37 injected in left groin.

*Oct. 25, 1928:* Tuberculin test strongly positive.

*Nov. 12, 1928:* Right kidney injected with approximately 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*Nov. 14, 1928:* Biopsy of right kidney. Large numbers of dense hyaline casts were found in the renal tubules. The tubular epithelium showed only mild degeneration. Focal periglomerular lymphocytic infiltration was frequently found. There was no necrosis or vascular damage. There was some perirenal suppuration.

*Jan. 9, 1929:* Tuberculin test weakly positive.

*Jan. 17, 1929:* Left kidney injected with 35 mg. of coagulated tuberculin protein.

*Jan. 19, 1929:* Biopsy of left kidney. Sections revealed proteinuria, but no casts and no inflammatory changes. 4 mg. of H 37 injected in right groin.

*Feb. 20, 1929:* Tuberculin test positive.

*Feb. 27, 1929:* Right kidney (first injected on Nov. 12, 1928) exposed and biopsy taken. 25 mg. of coagulated tuberculin protein injected. Biopsy section revealed no changes except a few minute spots of slight lymphocytic infiltration (Fig. 1).

*Mar. 1, 1929:* Goat killed. In the right kidney (injected February 27 and previously on November 12) an enormous number of hyaline and waxy casts were seen (Fig. 2). Very little degeneration of the tubular epithelium was apparent. No vascular damage or necrosis was seen, and inflammatory changes were absent. The left kidney (injected January 17) was practically normal.

*Goat 7. Normal*

*Sept. 10, 1928:* Tuberculin test negative.

*Oct. 10, 1928:* Left kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*Oct. 12, 1928:* Biopsy of left kidney. Sections revealed almost no changes. A very few casts were found.

*Pig 3. Normal*

*May 22, 1928:* Right kidney injected with 20 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*May 24, 1928:* Biopsy of right kidney. Little change seen. One area of tubular necrosis from arterial obstruction was seen. No signs of inflammation were found. Sudan III stains showed no fatty degeneration.

*June 4, 1929:* Tuberculin test negative.

*June 5, 1929:* Left kidney injected in similar manner to right on May 22.

*June 7, 1929:* Operation for biopsy of left kidney. Kidney was found completely infarcted and was removed.

*Aug. 25, 1929:* Pig killed. Sections showed the right kidney to be practically normal. A few cortical scars from old infarction were seen, and an occasional fusion of glomerular tuft and capsule was noted.

*Pig 4. Normal at First. Later Tuberculous*

*May 24, 1928:* Right kidney injected with 20 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*May 27, 1928:* Biopsy of right kidney. Sections showed almost no change. A very few leucocytic casts were found.

*June 4, 1928:* Tuberculin test negative.

*June 7, 1928:* Left kidney injected in similar manner to right on May 24.

*June 9, 1928:* Biopsy of left kidney. Extensive gross infarction found. Sections showed extensive thrombosis, infarction and a good many polymorphonuclear leucocytes in the infarcted areas. Sudan III sections showed fatty degeneration limited to the infarcted areas.

*July 15, 1928:* Pig injected in groin with 2 mg. of bovine type tubercle bacilli. Marked enlargement of the regional lymph nodes developed in the following weeks and then subsided.

*Aug. 24, 1928:* Right kidney injected with 5 mg. of coagulated tuberculin protein in usual manner.

were seen. No glomerular changes could be found. Sudan III stains revealed profound fatty degeneration of the tubules.

*July 11, 1929:* Left kidney injected in similar manner to right on June 10. A gasping respiration developed and the pig died just as the injection was finished. Autopsy: fibrocaceous tuberculosis of the inguinal lymph nodes regional to the site of infection with tubercle bacilli. No other tuberculosis. The right kidney, injected June 10, appeared normal except for a few patches of interstitial lymphocytic infiltration and a slightly increased cellularity of the glomeruli. The left kidney, injected just before death, was normal.

### *Pig 2. Tuberculous*

*May 1, 1929:* Infected in groin with 2 mg. of human type tubercle bacilli.

*June 4, 1929:* Tuberculin test positive, moderate degree.

*June 12, 1929:* Right kidney injected with 10 mg. of coagulated tuberculin protein suspended in salt-citrate solution.

*June 14, 1929:* Biopsy of right kidney. Small regions of infarction from vascular occlusion were found. Outside of the infarcted regions a good many hyaline and a few leucocytic casts were found in the tubules. The glomeruli appeared normal. A few patches of interstitial lymphocytic infiltration were seen. There was some perirenal suppuration, presumably from infection at the time of operation. Sudan III stains revealed a profound fatty degeneration of the tubular epithelium.

*June 19, 1929:* Left kidney injected in similar manner to right on June 12. As in the case of Pig 1 the animal collapsed and died just as the injection was finished. Autopsy: fibrocaceous tuberculosis of the inguinal lymph glands regional to the site of infection with tubercle bacilli. No other tuberculosis. The right kidney (Fig. 3), injected June 12, was the seat of a marked interstitial nephritis, with intertubular edema, a profound interstitial lymphocytic infiltration, and many casts formed from polymorphonuclear leucocytes in the tubules. Leucocytes were numerous in the glomeruli, which were otherwise normal for the most part, but occasionally showed a slight proliferative change. The left kidney, injected just before death, showed no change except some protein precipitate in the tubules.

*Summary of Results in Swine*

Tuberculous swine proved only moderately skin sensitive to tuberculin. Injection of tuberculin protein into the renal artery in tuberculous swine, however, produced much more marked changes than occurred from similar treatment of either monkeys or goats. The lesions produced with the dosage of tuberculin used (10 mg.) were not as severe as those previously reported in swine following similar injection of large quantities of tuberculin protein (30-100 mg.). In particular the glomerular lesions noted in the former investigation were not repeated. On the other hand quite similar interstitial changes occurred. As in the former study, recovery from the lesions produced, with restoration practically to normal, occurred in the course of a few weeks after the injury. In more detail the results in swine were as follows: Renal arterial injection of suspended coagulated tuberculin protein in two tuberculous swine caused profound fatty degenerative changes in the tubular epithelium with hyaline and leucocytic casts. In one of these there was marked and in the other moderate interstitial infiltration with cells of inflammation. A second renal arterial injection, in the opposite kidney, led to abrupt exitus on the operating table in each of these animals (compare with asthma and shock in tuberculous animals of first investigation following injection of tuberculin protein). Two normal control pigs, similarly injected, showed no changes except lesions obviously the result of vascular thrombosis or embolism, a type of accident which occurred occasionally throughout the whole series of animals in this study.

## GENERAL SUMMARY

Distinct renal allergic responses were secured on the injection of tuberculin protein into the renal arteries of tuberculous monkeys, goats and swine. The allergic nature of the response was established by the fact that similar injection into normal controls did not cause injury (except such as occurred from vascular occlusion). In the monkeys the lesion produced was purely degenerative, in the goats chiefly degenerative but occasionally inflammatory, and in the swine degenerative and of a more inflammatory character than in the goats. The inflammation in the goats and swine took the

Oct. 11, 1928: Pig killed. Autopsy: fibrocaseous tuberculosis of lymph nodes regional to site of infection with tubercle bacilli. No disseminated tuberculosis. Right kidney smaller than left. Sections of each kidney revealed no significant microscopic changes.

### *Summary of Results in Monkeys*

Skin sensitiveness to tuberculin in the tuberculous monkeys of this experiment, infected intraperitoneally with tuberculosis, was low. Likewise only mild injury of the kidneys of these tuberculous monkeys could be produced by injection of a suspension of tuberculin protein containing particles of a sufficient size to obstruct glomerular capillaries. Aside from the occasional abrupt necrosis resulting from vascular thrombosis subsequent to injection of the artery, only mild degenerative changes with the production of a few casts, were seen in the tuberculous monkeys of this series, and inconstantly in these. In no case were the inflammatory changes characteristic of the tuberculin skin reaction in sensitive animals found. No changes were produced in normal animals.

### *Summary of Results in Goats*

Goats infected subcutaneously with bovine type tubercle bacilli rapidly succumbed with extensive pulmonary miliary tuberculosis. One goat out of three so infected, which lived long enough for further experiment, proved strongly skin sensitive to tuberculin and developed a moderate acute interstitial nephritis, with pronounced tubular degenerative changes, on arterial injection of the kidneys with tuberculin protein. Three goats injected subcutaneously with human type tubercle bacilli gave strongly positive tuberculin skin tests five weeks later. Renal arterial injections of the kidneys of these animals resulted in slight tubular degenerative changes in one, acute degenerative changes on the second of two injections, with slight inflammation, in a second, and acute tubular degeneration with many hyaline and waxy casts in a third. A second injection of tuberculin protein into the same kidney of the latter animal, three months later, led to the production of an enormous number of dense hyaline casts with no appreciable inflammation and little apparent tubular degeneration. Injection of tuberculin into normal control goats led to no changes, except those due to accidental vascular injury.



## DESCRIPTION OF PLATE

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### PLATE 96

- FIG. 1. Right kidney of tuberculous Goat 6 on Feb. 27, 1929. Essentially normal three and one-half months after renal tuberculin reaction (Nov. 12, 1928).  $\times 125$ .
- FIG. 2. Right kidney of tuberculous Goat 6 on Mar. 1, 1929, two days after injection of tuberculin protein into right renal artery. (Fig. 1 shows the appearance of this kidney before the injection, as revealed by biopsy.)  $\times 135$ .
- FIG. 3. Right kidney of tuberculous Pig 2 seven days after injection of tuberculin protein into right renal artery. Biopsy five days previously had revealed a more acute reaction.  $\times 240$ .

form of an interstitial nephritis. The glomerular changes observed in a former investigation in which larger dosage of tuberculin protein was used, were not produced. The intensity of renal tuberculin reaction did not parallel the intensity of cutaneous reaction in this series of animals.

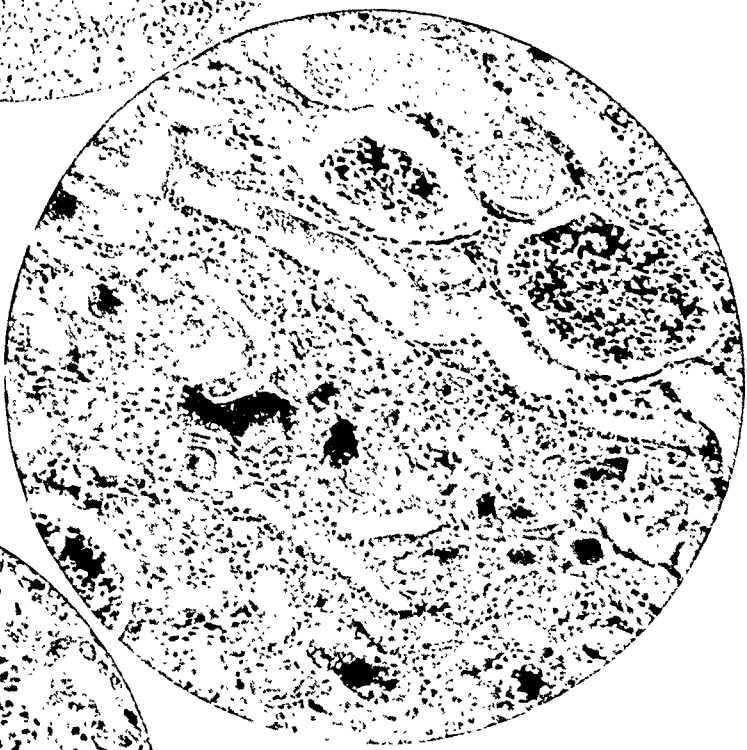
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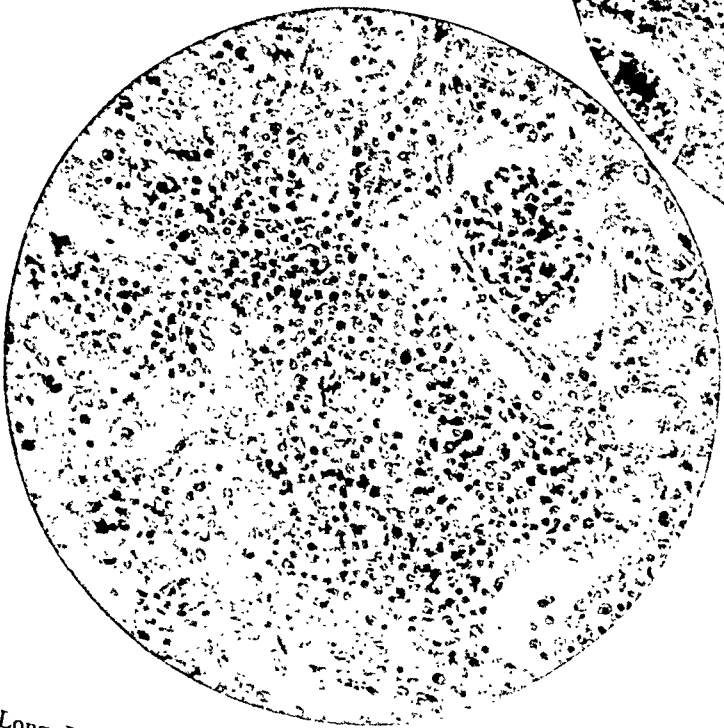




1



2



3

Long, Huggins and Vorwald

Intrarenal Arterial Tuberculin Injections

## CASE REPORT

*Clinical History:* A.D. (B. H., chronic cardiac valvular disease 2518 — 2nd Div.), an Italian male, 23 years of age, entered the hospital by ambulance on October 10, 1929, in a stuporous, semi-moribund state of acute cardiac decompensation.

*Present Illness:* No history was obtained from the patient. His brother stated that the patient had been ill for a long time with heart trouble and worked irregularly. The brother found the patient on the floor of his home and believed him to be almost dead.

*Physical Examination:* A young, fairly well developed, poorly nourished Italian male, extremely dyspneic and orthopneic with cyanosis of the lips and finger-tips, was lying propped up in bed. The respirations were shallow and rapid (24), temperature 100° F, and pulse 112. The patient was unable to talk and had a right facial weakness. There were petechial hemorrhages in the right conjunctiva and over the skin of the chest and arms. The mucous membranes were pale. The chest was thin-walled and the expansions were equal. The breath sounds over the anterior portions of the chest were harsh and loud with sibilant and sonorous râles predominating. Posteriorly, the breath sounds were harsh, vesicular, with numerous moist râles at both bases.

The apex beat of the heart was visible and palpable in the fifth and sixth intercostal spaces from the nipple to the anterior axillary lines. The heart was enlarged to the right as its border of dullness was 5.0, 5.0, 8.5 cm. to the right of the midsternal line in the second, third and fourth intercostal spaces respectively. The rhythm was totally irregular. The apex beat was 132, and the radial pulse 112 per minute, a pulse deficit of 20. There was a marked systolic thrill at the apex. A short presystolic murmur followed by a prolonged blowing murmur, maximum at the apex, and replacing the first sound was heard over the precordia. The pulmonary second sound was greater than the aortic second sound. The pulses were equal, small and totally irregular.

The liver edge was not felt. The right upper and lower extremities lay limply at the patient's side completely paralyzed. Deep tendon reflexes were present on both sides. No Babinski.

*Laboratory Findings:* The urine was yellow, acid, specific gravity 1.018, with a slight trace of albumin, but no sugar.

# MARKED DILATATION OF THE LEFT AURICLE OF THE HEART \*

## REPORT OF A CASE

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Marked dilatation of the left auricle of the heart is a relatively rare finding at the autopsy table. This is the report of a case coming to autopsy at the Bellevue Hospital.

## REVIEW OF LITERATURE

The original clinical and pathological descriptions of extreme left auricle dilatation were contributed by Owen and Fenton<sup>1</sup> in 1901. Their patient presented symptoms of right pleural effusion for which a thoracentesis was performed and pure blood obtained. At autopsy the left auricle was markedly dilated and contained 30 ounces of blood, as compared with the normal content of 2 to 4 ounces. Shaw<sup>2</sup> reports a heart whose left auricle held 30 ounces. Emanuel<sup>3</sup> reports a heart whose left and right auricles held 40 ounces and 20 ounces respectively. In East's patient<sup>4</sup> the left auricle held  $1\frac{1}{2}$  pints (23 ounces) of blood and the auricular wall was so thinned that no muscle could be detected.

Schott<sup>5</sup> describes the pathogenesis of left auricular dilatation and demonstrates by orthodiagrams the progressive enlargement of the auricle.

Bordet<sup>6</sup> conducted X-ray studies in a large series of cases showing mitral stenosis and found the left auricle dilated to the right of the right auricle in 5 per cent of the cases.

Bedford<sup>7</sup> states that early diagnosis of left auricular dilatation beyond the right auricle is possible only by X-ray, but that later suggestive signs and symptoms of its presence may appear.

Bach and Keith<sup>8</sup> describe a patient with active rheumatic fever who came to autopsy and presented a dilated left auricle which widened the interbronchial angle and compressed the left bronchus.

\* Read before the New York Pathological Society, November 21, 1929.  
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ments are: tricuspid valve 12.5, pulmonary valve 6.8, mitral valve 4.0, aortic valve 6.2, left ventricle 1.2, and right ventricle 0.3 cm.

The other organs are not remarkable.

*Anatomical Diagnoses:* Stenosis of mitral and aortic valves, hypertrophy and marked dilatation of left auricle, mural thrombus left auricle.

### MICROSCOPIC EXAMINATION

*Heart:* The ventricular musculature is normal. The nuclei of the auricular musculature are large and the fibers are hypertrophied. The muscle fibers immediately below the endocardium are necrotic and have lost their nuclei. The auricular myocardium is infiltrated with lymphocytes.

*Lungs:* Many pigment-containing endothelial leucocytes are present.

*Liver:* Pigment present in the cells about the central veins.

The other organs are not remarkable.

### DISCUSSION

The auricular dilatation, as described here, is associated with mitral stenosis of rheumatic origin. Many cases show more marked mitral stenosis with little or no auricular dilatation. Emanuel<sup>3</sup> believes that the auricular dilatation is due to auricular myocardial damage. To prove this point he describes a heart in St. Bartholomew's Hospital Museum,<sup>9</sup> whose left auricle is markedly dilated yet the mitral valve admits three fingers. A heart in the University College Hospital<sup>10</sup> is similar and the mitral valve is only slightly thickened. Bach and Keith<sup>8</sup> present a heart with marked left auricular dilatation whose mitral valve admits three fingers. This case, however, as most cases cited in literature, presents a pericarditis with the valvular lesions. This reaction is symbolic of a pancarditis which includes the myocardium of the left auricle. Increased intra-auricular pressure through an incompetent mitral valve plus a damaged myocardium will probably account for marked left auricular dilatation.

The blood non-protein nitrogen was 45, and the sugar 75 mg. per 100 cc.

The Wasserman was negative. Cytological studies of the blood were not conducted.

*Progress Notes:* The patient became more cyanosed, temperature progressed to 103° F and he died thirty-two hours after entering the hospital.

*Clinical Diagnoses:* Chronic cardiac valvular disease (rheumatic) with auricular fibrillation, embolism of the left internal capsule, and subacute bacterial endocarditis (?).

### AUTOPSY REPORT

The autopsy was performed on October 11, 1929 (Accession Number 14736).

The body weighs 115 pounds and measures 175 cm. in length. The chest and arms contain numerous small petechial hemorrhages. There is no edema present.

*Pericardium:* The sac is markedly distended and extends 8 cm. to the right and 7 cm. to the left of the midsternal line at the level of the diaphragm. The cavity contains 150 cc. of straw-colored fluid. The pericardial surfaces are smooth, glistening and contain no adhesions.

*Heart:* Weight 540 gm. The left auricle is markedly dilated and its right border extends 8 cm. to the right of the midclavicular line in the region of the fourth rib. The lung tissue is compressed. The left border of the left auricle lies in the arch of the aorta. The left auricle has a capacity of 593 cc. of fluid (after fixation). The right auricle is slightly dilated. The auricular appendages are natural. Section through the left auricle shows an increase in thickness of the wall. The auricular endocardium contains a thin mural thrombus covering most of the surface. The opened left auricle (circumference) measures 25 cm. laterally and 15 cm. (circumference) above the mitral valve. The ventricular myocardium is apparently not hypertrophied.

The mitral valve is stenosed to a slit-like opening whose base is calcified and whose opening is 2.2 cm. in length and will not admit the smallest digit. The leaflets of the aortic valve are adherent, slightly thickened and the orifice is stenosed. The cardiac measure-



## DESCRIPTION OF PLATE

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### PLATE 97

FIG. 1. Dilated left auricle contains a thin mural thrombus. The mitral valve is stenosed and calcified.

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Three cases of the occipital type are described in the literature. (The report of a fourth case belonging to this group is not reliable.)

Most of the reports of this congenital anomaly belong to the older literature, and quite frequently the descriptions are somewhat unsatisfactory. In general, however, a review of the literature gives us the following picture: craniopagus is a condition usually incompatible with life, although the first case is reported to have lived ten years. This is the exception, however. Most of the cases, even if born at full term, are stillbirths and at most live only a short time, several hours or a few days. The malformation may be restricted to the skull and its contents, or may be accompanied by anomalies in other parts of the body. The examination of the central nervous system has in the past been restricted to the gross anatomy. Usually the brains are described as being separate. Histological studies have been omitted, the reasons probably being that, first, most of the cases have been reported in the older literature; second, in a number of the more recently described craniopagi, the brain is said to have been in a state of decomposition and not suitable for histological examination. In the case which I wish to report the central nervous system of one of the children was quite well preserved, and detailed histological examination was possible. It is because of this, therefore, that the case is presented.

The two children forming the craniopagus belonged to triplets which were born at full term. The mother, a primipara, was physically and mentally normal, and as far as is known there was nothing remarkable in the family history. The third member of the triplet was born dead, and I had no opportunity to examine it. The craniopagus lived for five days and the postmortem was performed several hours after death. As is seen from the picture (Fig. 1) they give the impression of full term infants. The hair and nails were well developed, and there were no malformations in any other part of the body.

The right parietotemporal part of the skull of one of the infants was grown together with the right parietal region of the other, so that the two children faced opposite directions, one facing left, and somewhat upwards, the other looking to the right. We shall call the first one "A," and the second one "B."

# HISTOLOGICAL STUDIES ON THE BRAIN OF A CRANIOPAGUS \*

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## INTRODUCTION

Craniopagus is one of the most infrequently occurring symmetrical malformations. In the literature only twenty-two cases have so far been reported, the first one dating back to a report by Sebastian Münster<sup>1</sup> in 1495.

Three types of craniopagus are generally recognized according to the part of the skull that is involved: first, the frontal; second, the occipital; third, the parietal regions. The first type occurs most infrequently, and up until now only three cases have been reported (one each by Münster,<sup>1</sup> Baer<sup>2</sup> and Warschauer).<sup>3</sup> The one reported by Münster is the most remarkable in that the children lived for ten years. They were six years of age when they first came under the observation of Münster in 1501. Baer's report (1845) consists of a short description of a museum specimen with a statement that "the brains communicated through an opening in the frontal bones." Warschauer's description was published in 1909. In his case the faces of the children were quite well developed up to the eyebrows. The left sides of both faces were somewhat smaller than the right. There was no further study of the brains, the reason being, as stated by him, that the tissues were "decomposed." The general structure of the two brains, as far as could be seen, was asymmetrical, and there was no definite dural fold between the two. The falx cerebri seemed to be pushed over to the left, making the space containing the right cerebral hemisphere about twice as large as the left. The brains were "probably separate."

The number of parietal craniopagi reported is fourteen. Twelve of these were collected by Ahlfeld<sup>4</sup> in his monograph in 1882. Two cases were added recently (Kissinger<sup>5</sup> in 1908, and Kafka<sup>6</sup> in 1920). The case which I wish to present also belongs to this group, making a total of fifteen.

\* Received for publication March 20, 1930.

calloso-marginal fissure, but no definite gyri can be traced. The left hemisphere is smaller than the right, but its structures are better defined. Here one can recognize the central sulcus, the Sylvian fissure and the inferior frontal sulcus. A number of smaller convolutions can also be seen, but are not identified. On the median surface the calcarine and callosal marginal fissures can be recognized; here too, there are a number of less well identified gyri. Although the general outline of the structures is very much like those found in the fetal brain, they differ somewhat from the full term infant, and are more like those present in a seven or eight months fetus.

*Cerebellum:* The cerebellum is asymmetrical, the left hemisphere being larger than the right (Fig. 2). It is only partially covered by the cerebrum and is in a developmental stage of the type found in a fetus of seven or eight months. The vermis and the two hemispheres can be clearly differentiated.

A frontal section through the brain at a level midway between the tips of the frontal and occipital lobes shows the following: The cut surface of the section is white; the cortex and white matter can be differentiated with difficulty, whereas the basal ganglia are fairly well outlined and apparently of normal contour; the corpus callosum does not show anything remarkable; in the unusually narrow white matter, several small foci of softening can be seen. The left lateral ventricle is of comparatively normal size and shape. The right lateral ventricle, however, is greatly enlarged laterally although it has a small vertical diameter. Sections through the cerebellum and medulla show the fourth ventricle to be asymmetrical, the left side being larger than the right; other abnormalities of significance are lacking.

#### HISTOLOGICAL EXAMINATION

*Left Hemisphere:* Of the two hemispheres this is the better preserved and the more highly differentiated. The cyto-architecture here differs in a great many respects from that of the new-born child. The predominant picture in the cortex is that of a six layer type (see Fig. 3); the first layer is rather poorly supplied with cell elements. The second is dense but narrow, and gradually passes into the third layer which becomes quite distinct from the second as it is definitely poorer in cells. The fourth (the inner granular layer) is quite well defined and compact, in contrast to the fifth which again

## SKULL OF CHILD "A"

The base of the skull is asymmetric. The left side is practically normal. The three fossae are well recognizable, although they are somewhat accentuated, corresponding to a similar enlargement of the brain. The frontal, parietal and occipital bones of this side are essentially normal. The right side of the skull presents a different condition. The anterior and middle fossae are confluent, forming one large depression in which the markedly deformed right hemisphere lies. The borders of the posterior fossa can not be followed. The right frontal bone is somewhat smaller than the left. All that remains of the right temporal bone is a very narrow strip at the base of the skull, and the parietal bone is entirely absent, leaving a large defect. The dura shows no malformations except for an opening in the parietal region corresponding to the defect of the skull. The cranial bones of Child "B" are normally developed with the exception of the right parietal bone in which a large defect is present. The base of the skull is quite well developed. It is through this defect that the right hemisphere of Child "A" extends into the cranial cavity of Child "B." The dural defect of "B" is similar to that of "A." The two brains, therefore, are separate units. The leptomeninges of the two brains are also separate and show no defects.

Of the two brains, that of Child "B" has been destroyed by the growth of the brain of Child "A" to such an extent that it is impossible to study it. Only a small portion of the occipital lobe of the compressed brain could be histologically examined. The brain of Child "A," however, is well preserved and presents the following features (see Fig. 2).

*Cerebrum:* The right hemisphere is larger and the gyri fewer than the left. The gyri are flat and hardly recognizable, and most of the sulci are absent, thus making it very difficult to differentiate between the various lobes. Even the Sylvian fissure can not be made out with certainty. It extends down into the base of the brain and can be followed there through the whole width of the cerebrum. Another deep fissure along the distorted frontal pole may represent the central sulcus. The temporal pole can not be clearly outlined. Excepting the above mentioned Sylvian fissure, no other identifications can be seen at the base. The olfactory tract and bulbs are well developed. On the median surface one can only recognize the



to proliferation. Occasionally protoplasmic elements can be seen, such cells containing some greenish pigment. It is interesting to note that no gitter cells are found anywhere, although as it was mentioned above, there were occasionally small foci of softening in the white matter. The vascular system shows a mild proliferation. The endothelial cells are somewhat swollen and the course of the vessels shows a tendency to tortuosity. On frequent occasions one finds a fine black pigment deposit in the vessel walls. Unfortunately, no frozen sections could be successfully made, as the tissues were too soft even after a prolonged fixation in formalin. The section from the occipital lobe of the brain of Child "B" was the only part that could be histologically examined, showing the same picture as the left hemisphere of Child "A."

### DISCUSSION

In our attempt to determine the probable stage of development which has been reached by the central nervous system here described, we may be guided by the observations made in the cases of developing human beings. According to His,<sup>7</sup> the cortex remains uniform showing no laminar structure until the fifth month of intra-uterine life. Then a gradual grouping of the neuroblasts into layers occurs. First, the fifth and sixth layers appear, and these are followed by the differentiation of the second, third and fourth. This process generally extends over the period from the sixth to eighth month, at the end of which time the six layer type is definitely recognizable. The different fields show some difference as to the time when this stage is reached. As this development goes on the agranular type of structure appears. As we know, the agranular type is the product of a change in the six layer type where the inner granular layer disappears. As an example of this type we can take the anterior central region of the new-born child. The appearance of the agranular type takes place after the formation of a six layer cortex, but does not appear at the same time in all fields. It is because of this fact that we can differentiate the different stages of development by the histological picture. In our case, for instance, in the left hemisphere we found that although pyramidal cells have appeared in the central convolution, they were as yet interspersed with a fourth granular layer; in other words, we were dealing there with a stage when the six layer type was gradually changing into an agranular type. This

has a rather sparse cell content. The sixth layer is not quite as definite, merging into the white matter, but nevertheless well recognizable. The agranular type of cortex is not quite as distinctly represented but is nevertheless definitely recognizable. Here also six layers can be distinguished. The first has a poor cell content as contrasted with the richly cellular second layer. This latter is wide and most probably represents a fusion of the third, fourth, fifth and sixth layers. The cortex is very well demarcated from the white matter. The line of demarcation between the granular and agranular types is also quite distinct. Outside of these two predominating types, some other fields can be more or less distinctly recognized. Thus, for instance, the anterior central cortical region can be identified by the presence of the giant pyramidal cells. Then again, the calcarine area is characterized by the widening of the fourth layer. The point of highest differentiation, and a development which comes nearest to that of a new-born child is reached in the allo cortex where the uncus and the cornu ammonis are easily recognized and do not differ from that of the full term infant.

*Right Hemisphere:* The architecture is very much disorganized by the intense distortion. The calcarine formation is entirely absent. It is practically impossible to differentiate the various fields, all of them being represented by a more or less uniform six layer cortex. The development of the allo cortex is much inhibited and even the usually well recognizable cornu ammonis can not be distinguished. The different convolutions are mostly only rudimentary, rather large, and flat (see Fig. 4). The different cortical layers are unevenly distributed showing at various places indentations into one another. Their cell content also is irregularly distributed. The whole picture is that of a distorted compressed brain tissue.

*Basal Ganglia:* The pallidum has the most normally developed architecture. Its parenchyma varies little from that of a new-born child. The striatum, however, does not show as highly a differentiated development. It contains numerous cells. The large ganglion cells are well represented. The small ones, however, still bear a resemblance to neuroblasts. A similar resemblance to the neuroblast type is found in the cells of the thalamus. The pons, cerebellum and cord show practically no difference from those found in the full term infant. The sections stained with Weigert's method reveal no myelin sheaths. The neuroglia in general disclose no tendency

4. The general outlines of the brain are like those in a seven or eight months old fetus.

5. Histologically both the granular and agranular six layer types of cortex are present.

6. In the precentral region the fourth layer is still granular in structure, which corresponds to the development of the eighth month of intrauterine life.

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## DESCRIPTION OF PLATES

### PLATE 98

FIG. 1. The heads of the two infants are united in such a way that the right parietotemporal region of the one on the right is grown together with the right parieto-occipital region of the one on the left.

FIG. 2. Brain of the Child "A." The right hemisphere is larger than the left. The convolutions show pronounced malformations. The right occipital lobe is absent. The left hemisphere is better developed. The cerebellum is asymmetric, the right lobe being much smaller than the left; the cerebellum is not covered by the cerebrum. The spinal cord is normal.

would lead us to place the probable height of development of the brain at the eighth month of intra-uterine life.

The developmental stage reached by the brain, as well as the development of the rest of the body would lead one to assume that the fusion of the two skulls was the result of a late malformation (this would agree with Schwalbe's <sup>8</sup> views on the subject). All our observations point to the fact that we are dealing here with two distinct embryos, each one of which in itself has no tendency for developmental anomalies. The whole central nervous system seems to have started in a normal process of development, and has progressed along this line until the rapid increase in size due to the formation of the convolutions which have come up against the obstacle of the limited space available. It was here that apparently a purely mechanical compression interfered with the normal rate and direction of development and brought about the type of structure that we have described. We can see that the pressure phenomena begin to influence the development of the brain about the time of the convolutional formations because the gyri that are found here are simple, smooth and extraordinarily flat. The sulci are shallow and in places pushed out of their normal position. In some places, whole regions are either destroyed or compressed to such a degree that they can not be recognized. All of this would speak for the appearance of a marked scarcity in space at the time when development was at its highest. It is quite possible too that the fusion of the skulls appears rather late, and that at an earlier stage the two were distinctly separate. This is rendered especially probable because of the fact that different parts of the skulls have fused with one another, the right parietotemporal of one with both parietal bones of the other. This again has also been noted by Schwalbe. Just why the brain of the one child has been damaged so much more than the other can not be answered.

#### SUMMARY

1. The case described belongs in the group of craniopagus parietalis.
2. The malformation is confined to the skull and brain.
3. The brain of the child "A" is asymmetrical and shows pronounced malformation of the gyri and sulci which makes the identification of the latter difficult. Many sulci are absent.

PLATE 99

FIG. 3. Shows clearly the six layer type of the fetal cortex.

FIG. 4. Six layer type clearly visible. Sulci shallow and primitive.

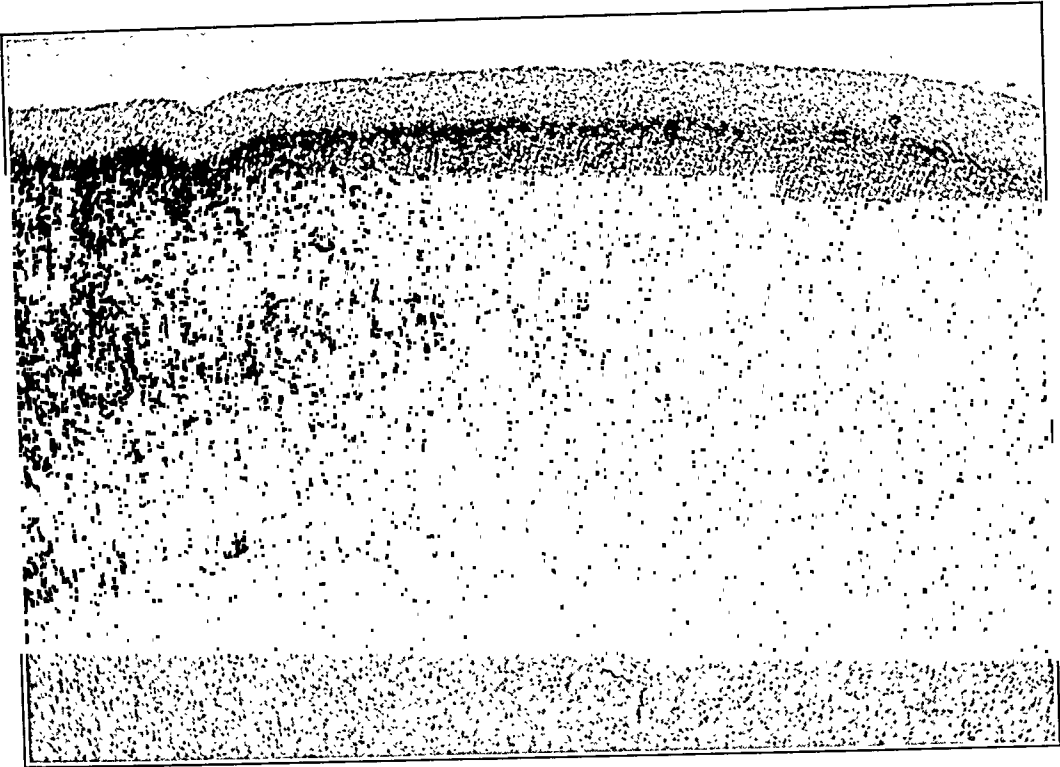


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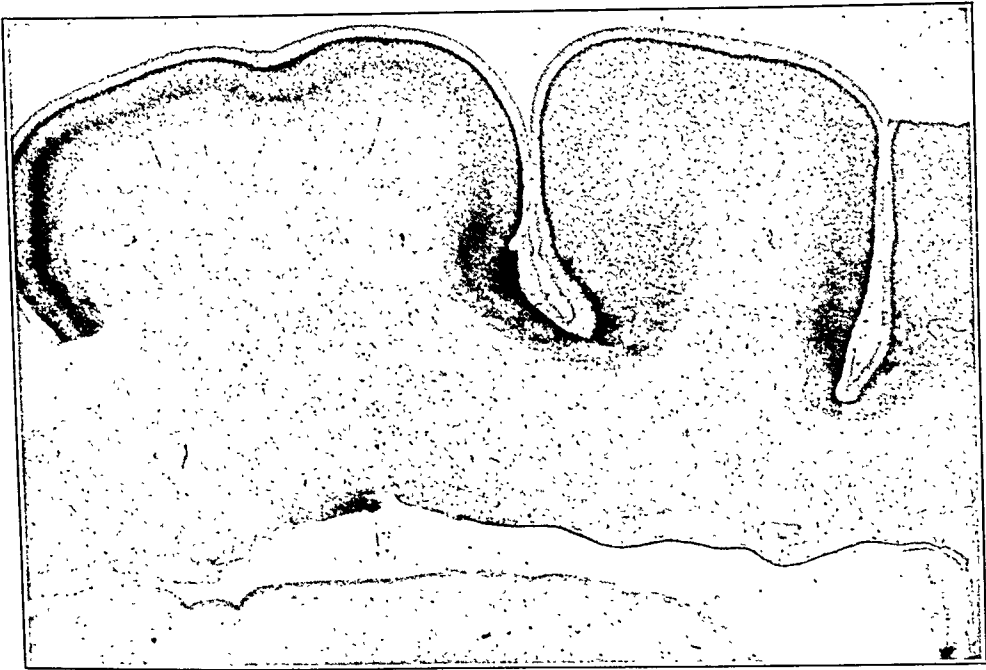


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and in addition a marked degree of focal necrosis was present in the liver.

The heart weighed 290 gm. and measured 12.4 by 13.5 by 5.2 cm. The apex was quite sharp and was formed chiefly by the tip of the left ventricle. The epicardial fat was moderate in amount. The coronary arteries were not palpably thickened. The right heart contained a considerable amount of postmortem clot. The valvular orifices measured: tricuspid 13.5, pulmonary 6.8, aortic 6.5, mitral 10.7 cm. The valve curtains throughout were thin, translucent and pliable. Two small fatty plaques were present in the free cusp of the mitral valve. The undefended space in the septum was anomalous in its appearance, there being a marked aneurysmal pouching extending from the left ventricle to the right auricle. It arose 9 mm. below the common point of attachment of the right and left posterior aortic valve cusps to the aortic wall. It was roughly circular in contour, and measured 2 by 2 cm. in width and 1.8 cm. in depth. Several fibrous trabeculae extended from the periphery of the pouch toward its central portions. These were of approximately the same thickness as the ring of fibrous material about the periphery, but they were considerably thicker than the central areas of the pouch, which were glistening, thin-walled and translucent. The aortic valve cusps were quite competent. The sinuses of Valsalva presented no abnormalities. On looking through the aortic ring into the heart, the aorta was seen to be directed toward the right ventricle. The muscular septum protruded into the aortic vestibule and the membranous septum was almost horizontal instead of vertical. The endocardium of the left ventricle was smooth and glistening. The aneurysmal pouching extended in a rounded manner through the medial cusp of the tricuspid valve at its junction with the anterior cusp. The latter of these formed the left lateral boundary of the pouch, which measured 2.3 by 1.8 cm. Its surface was smooth and glistening. The tricuspid valve cusps were thin and pliable and no thickenings of their free margins were seen. The foramen ovale was patent in a slit-like manner along the anterior margin of the fossa ovalis. The wall of the left ventricle measured 1.7 cm. in thickness, and that of the right ventricle measured 5 mm. The cut surface of the heart muscle was deep pinkish red in color. The surface was glistening in appearance and no whitish streaks were seen. The coronary arteries arose in normal position. They were thin-walled throughout, and presented no areas of atheroma.

# CONGENITAL ANEURYSM OF THE INTERVENTRICULAR SEPTUM \*

## REPORT OF TWO CASES

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Congenital anomalies of the heart are of not infrequent occurrence. Congenital aneurysms of the interventricular septum, however, are quite unusual, and when encountered are valued for anatomopathological studies rather than for any practical importance from a clinical standpoint. They are usually discovered incidentally at autopsy. If they give rise to symptoms during life, these are of such complicated nature that the diagnosis is rarely made correctly. The following two cases are of interest in that they were seen within one month of each other at autopsy, and neither gave any clinical evidence of its presence.

### CASE I

*Clinical History:* G. H., a young man 24 years of age was admitted to the medical service of the Toronto General Hospital on March 12, 1928, complaining of pain in the left chest, with chills and fever for three days. Nothing further of importance for this report was elicited in the history. The physical findings were those of a severe pneumonia of the left lower lobe along with some cardiac enlargement. The patient became progressively worse until his death four days later.

### POSTMORTEM EXAMINATION

The postmortem examination revealed a confluent bronchopneumonia in the lower lobe of the left lung with partial collapse of both right and left upper lobes. A marked fibrinous pleurisy was present on the surface of both lungs, and a moderate effusion was found in the pleural cavities. A Meckel's diverticulum was seen about two feet proximal to the ileocecal valve; no other abnormalities were found in the abdominal cavity. The left kidney and the spleen were the site of numerous, small infarcts, while healed miliary tubercles were found in the spleen and liver. These organs presented the usual picture of cloudy swelling associated with severe toxemias,

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attached to this rudimentary cusp. The aneurysmal sac was multilocular in appearance. Its surface was irregularly thickened by fibrous trabeculae running inward from its periphery. As in the previous case, there were translucent areas in its central portion. It measured 2.5 by 1.8 cm. as seen from the right ventricle. The tricuspid valve was apparently competent. There was a small fibrous band running across the cavity of the right auricle. The foramen ovale was closed. The pulmonary valve consisted of two cusps only. These were large, and one of them presented a fibrous band joining its free margin to the wall of the pulmonary artery at a point 1 cm. from the insertion of the base of the cusp into the wall of this vessel. This formed a rudimentary third cusp which measured 1 cm. in width. A small ridge was found in the base of the sinus immediately below this fibrous band. The width of the larger curtain was 4.5 cm., and the smaller curtain was 2.4 cm. The free margins of these curtains were slightly thickened and somewhat roughened. The aortic valve cusps were much larger than usual and presented numerous fenestrations. The right curtain measured 3.5 cm., the left 2.5 cm., and the posterior 2.9 cm. in width. The right and posterior cusps were fused in an irregular manner, but no thickening was present at their point of fusion. In looking through the aortic ring into the heart, the aorta was seen to be directed toward the right ventricle. The muscular septum projected into the aortic vestibule and the membranous septum was horizontal in direction instead of vertical, its floor being formed in part by the floor of the aneurysm. The mouth of the aneurysmal sac opened at a point 2.5 cm. below the common point of attachment of the posterior and right valve cusps. It measured 2.8 by 1.6 cm. in width, 3.8 cm. in depth and was somewhat oval in shape. It presented a multiloculated appearance, with small pouches extending outward from the largest one, which occupied the greater portion of the undefended space of the interventricular septum and projected into the right ventricle beneath the medial cusp of the tricuspid valve. Its surface was a glistening white color, and there was no evidence of a previous inflammatory process. The sinuses of Valsalva were very large, but normal in general contour, except that the base of the right sinus was formed by myocardial tissue. This sinus extended below the point of origin of the wall of the aorta for a distance of 5 mm. The mitral valve curtains showed a slight degree of diffuse thickening of their free margins, and the septal cusp presented a pouch measuring

## CASE 2

*Clinical History:* J. R., a man, aged 60 years was admitted to the surgical service of the Toronto General Hospital on March 1, 1928, complaining of difficult micturition and almost complete retention of urine. History and physical examination beyond the genito-urinary tract were negative. One month later, following the resection of a vesical diverticulum, the patient developed peritonitis and died.

## POSTMORTEM EXAMINATION

At autopsy a foul-smelling peritoneal exudate was found which had its source in the urinary bladder. In addition to congenital cardiac anomalies, the examination revealed a congenital absence of the gall bladder and cystic duct, together with abnormal lobation of the right lung, the presence of accessory spleens and supernumerary renal arteries. No noteworthy pathological changes were found elsewhere.

The heart weighed 365 gm. and measured 15.2 by 12.5 by 5.2 cm. The right heart was somewhat dilated, but the left ventricle was contracted. The heart presented two apices and the right border was sharply concave. The main apex was sharp and was formed by the tip of the left ventricle. The apex of the right ventricle stood out at right angles from the septum and formed a firm mass near the line of the interventricular groove. The epicardial fat was moderate in amount. There was a small, white, localized thickening of the pericardium of the left ventricle. The base of the right ventricle felt quite thick and projected outward from the septum for a distance of 12 cm. on the posterior surface, and 9.8 cm. on the anterior surface. The pulmonary arteries were very large, measuring 9.2 cm. in circumference. The superior vena cava was quite small, barely admitting an index finger. The cavities of the heart contained small quantities of postmortem clot. The valvular orifices measured: tricuspid 12.5, pulmonary 7.3, aortic 8.2, mitral 10.2 cm. The tricuspid valve curtains presented a slight amount of diffuse thickening of their free margins. The medial cusp of this valve was quite thin. A sacculated aneurysm occupying the region of the undefended space of the interventricular septum projected into the right ventricle for a distance of 2 cm. at the common point of attachment of the medial and anterior cusps. The valve curtain over the aneurysm was slightly thickened and a small projection was present, which simulated a fourth valve cusp. No chordae tendineae were

the right ventricle, through the undefended space which later became perforated.

The case reported by Guccione was somewhat akin to Case 1, but possessed certain features which necessarily place it in a separate class. A distinct funnel-shaped protuberance of the wall emerged immediately above the septal cusp of the tricuspid valve. The sharpened apex of the aneurysm was directed downward toward the ventricle and was partially covered by recent thrombi. On removing these, the remaining portion of the aneurysm presented a transverse opening, which could be penetrated by a probe. The protuberance was covered by thickened endocardium and was pasty in consistency. The aortic valves were markedly distorted by recent vegetations and old scars. Between the thrombi on the posterior cusp and the base of implantation of the anterior cusp of the mitral valve, a fissure was observed in the interventricular septum. On removing the clots filling this rupture, a funnel-shaped diverticulum was seen, which was also filled with thrombi. This corresponded with the swelling in the right atrium, immediately above the septal cusp of the tricuspid valve.

The etiology of these aneurysms is a matter of some disagreement. Guccione believes that the primary production of the aneurysm is due to endocarditis. This, he states, is usually an extension of the process from the mitral or aortic valve cusps, which is facilitated by their proximity to the membranous septum. Following erosion of the endocardium over the pars membranacea, the blood tends to infiltrate into the lower stratum of the septum, because the pressure is higher in the left ventricle than in the other cavities of the heart. Thus, Guccione concludes, they must be considered as "true dissecting aneurysms, not only because they are as a rule acute, but also on account of their evolution." Histological examination in his case further adds to the strength of his views. He considered that the aneurysm was due to recurrent chronic aortic endocarditis, with a terminal acute attack which resulted in ulceration of the sac and the subsequent death of the patient.

Mall and Goehring, on the other hand, consider that these aneurysms are the result of congenital malformations. The latter points out that it is difficult to understand how an aneurysm can penetrate the dense fibrous ring of the atrioventricular orifice, and burrow into the delicate fibrous tissue of the cusp of a fully developed heart. He suggests that they occur some time after the completion of the

1.5 by 1.3 cm. in diameter, which projected for a distance of 1.2 cm. into the left auricle. The coronary arteries arose slightly above the level of the aortic ring. Their mouths were large and funnel-shaped. They did not lie over the centers of the sinuses from which they arose, but were situated at the adjacent margins of the respective sinuses. These vessels were thin-walled throughout, presenting only one or two small, yellowish plaques beneath their intima. The wall of the left ventricle measured 1.8 cm. in thickness, and that of the right ventricle measured from 3 to 8 mm. The cut surface of the heart muscle was deep pinkish-red in color, showing numerous red stipplings. A localized area of fibrosis, measuring 2.2 by 1.5 cm. in diameter, was found on the lateral surface of the left ventricle near the tip. The wall of the ventricle at this point presented a small area of bulging, measuring 5 mm. in diameter. The large size of the aneurysm in this case, in the absence of any evidence of valvular incompetence, was remarkable.

#### DISCUSSION

Other cases of aneurysm of the interventricular septum have been reported by Mall,<sup>1</sup> McCallum,<sup>2</sup> Merkel,<sup>3</sup> Tate,<sup>4</sup> Guccione<sup>5</sup> and Goehring.<sup>6</sup>

Those of Mall and Merkel were similar in appearance and localization to our first case. In that of the former a cystiform aneurysm was present at the junction of the aorta with the left ventricle. It involved the membranous septum, burrowed into the anterior part of the medial cusp of the tricuspid valve, and projected into the right atrium. Merkel's case presented an aneurysm from the left ventricle into the medial cusp of the tricuspid valve, as well as a partially patent interventricular septum. On the other hand, those of McCallum and Tate more nearly simulated the findings of our second case. In that of the former the pars membranacea septi bulged into the right ventricle, forming a sacculated projection beneath the tricuspid valve. The mouth of this sac was seen just below the aortic valves. In that of the latter a trumpet-shaped tube of membrane was found projecting into the right ventricle, at the junction of the attached margin of the septal and infundibular segments of the tricuspid valve. On passing a probe along this hollow membranous tube, it was seen to communicate by a rounded opening with the lower part of the right cusp of the aortic valve. It appeared to have commenced as a left-sided saccular projection into

Guccione may have resulted from the superimposing of an endocarditis upon a congenital anomaly. Such anomalies are notorious as loci for infection.

The presence of associated anomalies in the heart and other organs in the second case is of great interest, and adds additional weight to the theory of the congenital nature of these aneurysms.

### SUMMARY

1. The clinical and pathological findings in two cases with congenital aneurysm of the interventricular septum are here reported.

2. The absence of clinical signs and symptoms in both cases, wherein marked distortion of the normal anatomy was present, is remarkable.

3. A critical study of our cases adds further evidence in favor of these anomalies being congenital malformations, rather than the terminal results of endocarditis.

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### DESCRIPTION OF PLATES

#### PLATE 100

FIG. 1. Left side of heart (Case 1). (A) Aneurysm. (B) Anterior aortic valve cusp. (C) Right posterior aortic valve cusp. (D) Left posterior aortic valve cusp.

FIG. 2. Right side of heart (Case 1). (A) Aneurysm bulging through medial cusp of tricuspid valve.

FIG. 3. Left side of heart (Case 2). (A) Coronary orifices. (B) Aneurysm. (C) Anterior cusp of mitral valve.

FIG. 4. Right side of heart (Case 2). (A) Medial cusp of tricuspid valve. (B) Aneurysm. (C) Rudimentary fourth valve cusp. (D) Anterior cusp of tricuspid valve. (E) Inferior cusp of tricuspid valve.

FIG. 5. Right side of heart viewed from below tricuspid valve (Case 2). (A) Aneurysm. (B) Pulmonary artery.

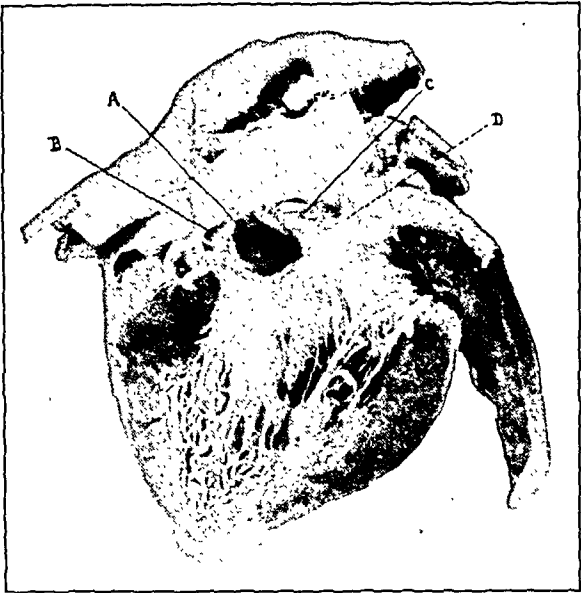
septum, but before the replacement of the musculature of the cusp by fibrous tissue.

Mall suggests that these aneurysms result from a failure of the inferior or fleshy portion of the interventricular septum to shift sufficiently far to the right. The aorta during the course of development of the heart, shifts from the right side of the heart to the left. According to Mall, after gaining its permanent position, the inferior septum blends with its right side, forms the membranous septum, and completes the wall between the left and right ventricles. This is in accordance with the views of Tandler<sup>7</sup> and Jordan,<sup>8</sup> who state that the membranous portion of the septum results from the fusion of the dorsal endocardial cushion dividing the atrioventricular canal, the proximal end of the aortic septum and the cephalic margin of the muscular interventricular septum. Mall points out that in the cases described by McCallum, Zahn, and Rokitansky, the muscular septum protruded into the vestibule of the aorta. This is also true of our cases. The aorta in the second case would seem to be communicating with the right ventricle rather than the left ventricle; this is also seen in the first case, but to a lesser degree. We may, therefore, conclude that in our cases, as in those of Mall and others, the aneurysm resulted from an embryonic arrest of development in which the inferior or muscular septum did not move sufficiently far to the right, but remained in the vestibule of the aorta. As a result of this misplacement the membranous septum developed in a horizontal plane instead of in its normal perpendicular one. This alone weakens it in every way, and with or without a possible defective texture could result in the production of an aneurysm. Normally the membranous septum lies below the tricuspid valve, but a slight distortion may displace the origin of the medial cusp on the membranous septum and result in the invasion of the valve, such as occurred in Case 1, whereas in Case 2 the aneurysm projected beneath the medial cusp of the tricuspid valve in the position where it would be expected.

The absence of any evidence of endocarditis in our cases, for one would hesitate to pronounce the thickenings of the mitral and tricuspid valve cusps in the second case as sequelae of a previous endocarditis, together with the failure to elicit any history of cardiac involvement, further strengthens the view that they are the result of congenital malformations, rather than the terminal results of endocarditis. Indeed, it seems possible that the acute case of



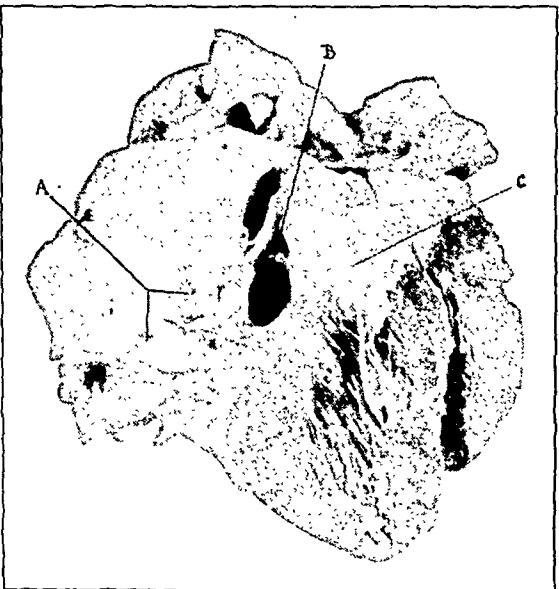




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low serum protein and a high albumin-globulin ratio. The non-protein nitrogen averaged 57 mg. per 100 cc. of blood.

The present paper deals with a detailed histological study of the lesions produced in the kidneys of rabbits by the administration of massive doses of irradiated ergosterol. The effect of these lesions upon kidney function, as demonstrated by the retention of nitrogenous products in the blood, was also studied.

### MATERIAL AND METHODS

Sixteen young rabbits, averaging about 2300 gm. in weight, were used. A preparation of "Irradiated Ergosterol, 1000 D"\* having one thousand times the antirachitic potency of cod liver oil was administered by stomach tube, in doses of from 3 cc. to 10 cc. at intervals of from one to four days, except for one experiment (No. 40, Table I), in which the average interval was five days. Three pairs of animals were killed on the fourth, sixth and eighth days respectively, in order to determine the early changes in the kidneys. The remaining ten were allowed to die from the effects of the irradiated ergosterol. A series of eight control animals received the non-active solvent oil used in the preparation of irradiated ergosterol employed, in similar doses.

From the control animals and the ten animals which received fatal doses of irradiated ergosterol, blood samples were taken at intervals and the non-protein nitrogen, urea nitrogen, creatinine and uric acid determined by the methods of Folin *et al.*<sup>10</sup> Hemoglobin was estimated by the Tallqvist method. The urine was tested for albumin, on the days when blood samples were taken, by the use of nitric acid.

Sections from the organs were fixed in an alcohol-formalin mixture (9 parts of 95 per cent alcohol to 1 part of 40 per cent formalin), in 10 per cent formalin, 95 per cent alcohol, Helley's fluid and in Zenker's fluid. A portion of the tissues fixed in formalin was embedded in celloidin and stained either with hematoxylin and eosin or by the silver method of von Kossa. Another portion of the formalin-fixed tissues was embedded in paraffin and stained with hematoxylin and eosin. In order to demonstrate fat, frozen sections of formalin-fixed material were stained with Sudan IV. Other sections

\* This preparation was supplied through the courtesy of Mead, Johnson & Co.

# RENAL LESIONS WITH RETENTION OF NITROGENOUS PRODUCTS PRODUCED BY MASSIVE DOSES OF IRRADIATED ERGOSTEROL \*

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## INTRODUCTION

Since the work of Hess,<sup>1</sup> Steenbock,<sup>2</sup> Hess and Windaus,<sup>3</sup> and others, showing that by the administration of irradiated ergosterol in extremely small doses it is possible to prevent or cure rickets, there has been much interest in the effect of this substance upon all conditions concerned with the metabolism of calcium. In addition, a number of investigators have studied the effects of very large doses of irradiated ergosterol upon experimental animals. The work of Pfannenstiel,<sup>4</sup> Kreitmair and Moll,<sup>5</sup> Klein,<sup>6</sup> Rabl,<sup>7</sup> and of Smith and Elvove<sup>8</sup> is especially noteworthy. They have shown that the administration of massive doses of irradiated ergosterol to rabbits and other susceptible animals leads to rapid loss of weight, cachexia and death. There is also a marked increase in blood calcium, and at autopsy extensive calcium deposits are found in the arterial walls, especially the aorta, in the heart muscle, stomach wall, lungs and kidneys.

The kidney lesions reported by previous investigators were chiefly in the parenchyma rather than in the blood vessels. Kreitmair and Hintzelmann<sup>9</sup> found, in rabbits, calcification in the membrana propria, around the convoluted tubules, also in the glomeruli, and sometimes in the vessels leading to them, with calcium deposits within the lumina of some of the straight tubules. Rabl described calcification of the basal membranes and epithelium of the tubules of the cortex in mice, with calcification of the vessel walls "in places." Smith and Elvove likewise found calcification and calcium casts in the tubules and in addition spoke of an "interstitial and glomerular nephritis." No calcium deposits were seen by them in the kidney vessels.

There is general agreement that the blood calcium rises markedly as a result of large doses of irradiated ergosterol. Klein noted also a

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living for a shorter period despite the fact that comparable doses of irradiated ergosterol were administered. This suggests that the time element is a very important factor in the production of the lesions. Regardless of whether or not gross changes were present, the capsule of each kidney was thin, stripped with ease and left a smooth glistening surface. Innumerable small, but easily visible, grayish brown areas (calcium) were studded throughout the cortex of the advanced cases. In the same cases a grayish brown concentric ring (calcium) approximately 0.1 cm. in thickness lay in the medulla, parallel to the curve of the cortex and 0.2 cm. medial to its inner margin. The cortex in all cases was of essentially normal thickness, and was well demarcated from the medulla.

Some sclerosis was observed in the renal arteries of the animals showing gross deposits of calcium in the cortex and medulla. On the other hand, the animals that died the most rapidly from ergosterol poisoning, but without gross changes in the cortex or medulla, showed little or no changes in the renal arteries. Rarely, slight thickening of the walls of the arteries was detectable when it was difficult to determine grossly if calcium deposition had occurred in the kidney.

The kidneys from the control animals, and from the animals receiving irradiated ergosterol for less than nine days, appeared normal in every respect.

#### MICROSCOPIC DESCRIPTION OF THE KIDNEYS

1. *Arteries:* The glomerular arterioles and the interlobular arteries (middle-sized and small arteries) in all the rabbits dying from irradiated ergosterol were sclerosed. The larger branches of the renal arteries usually appeared normal. The changes were focal in distribution and were almost entirely limited to the cortex and to the adjacent portion of the medulla. Hyalinization and calcification were the most characteristic changes in the walls of the affected vessels. The histological picture of the larger interlobular arteries was somewhat different from that of the glomerular arterioles. In the interlobular arteries the media was chiefly involved, whereas in the glomerular arterioles the media was principally involved, but often the other layers were hyalinized and fused. In the most damaged interlobular arteries the media and intima were fused in a manner comparable to the arterioles. The adventitia of the interlobular

of the same material were stained with methyl violet as a test for amyloid. The tissues fixed in Zenker's fluid were embedded in paraffin and stained with eosin-methylene blue. Weigert's elastic tissue stain was used on both formalin and Zenker-fixed materials. The calcium deposits were identified by their solubility in acid and by the following histological criteria, using principally formalin-fixed material: (1) When stained with hematoxylin and eosin the precipitated calcium appeared as a dark blue, coarsely granular material. (2) After treatment with a silver nitrate solution and counterstaining with a 0.5 per cent solution of basic fuchsin the deposits assumed a deep brownish black color.

## RESULTS

*Toxic Manifestations:* A demonstrable loss of the rabbit's body weight occurred within a few days after the administration of large doses of irradiated ergosterol was begun (Table I). Cachexia, loss of appetite, and oftentimes diarrhea developed as the irradiated ergosterol was continued. The hemoglobin showed a slight tendency to fall. The urine invariably contained a large quantity of albumin during the last day or two of life, and at the same time there was usually some retention of urine.

Of the control animals (Table II), four maintained a constant weight and two showed only a very gradual decline. The other two (Nos. 17 and 21), showed a considerable loss of weight and developed diarrhea. The urine showed small amounts of albumin on three occasions, a finding which is not unusual in apparently healthy rabbits.

## PATHOLOGICAL DESCRIPTION OF THE KIDNEYS

This report will be confined to the study of the kidneys of the twenty-four test animals, and the general pathological studies will be published subsequently.

*Gross:* The kidneys from the animals dying of irradiated ergosterol poisoning were found occasionally to be slightly reduced in size, but they always appeared normal in color, shape and consistence. After sectioning, however, definite macroscopic changes were seen in the cortex and medulla of the kidneys from the animals surviving the longest. These changes were not apparent in the animals

in the interlobular arteries than in the glomerular arterioles. However, strikingly different amounts of fat occurred in vessels of similar caliber and with a comparable degree of sclerosis. In the interlobular arteries the droplets were located chiefly in the media, while in the glomerular arterioles, the subintimal regions were especially rich in fat. These fat deposits were for the most part found in arteries showing marked calcification. However, some of the calcified vessels were free from fat, while occasionally there were scattered droplets of fat even in the walls of otherwise normal appearing vessels. Large amounts of fat filled the sites of obliterated lumina and the areas of necrosis within some of the vessel walls. The amount of fatty material was roughly proportional to the degree of general vessel involvement.

*Elastic Tissue:* There was marked thickening of the internal elastic lamina of many of the interlobular arteries. Thickening of the layers and splitting of the fibers was common. In general the external elastic lamina was normal. Occasionally, however, some of the individual fibers were irregularly thickened. The elastic tissue of the glomerular arterioles, when these were markedly involved, was indistinguishably fused with the adjacent portions of the wall.

2. *Veins:* The middle-sized and smaller veins showed changes similar to those in the corresponding arteries, but these changes were neither so numerous nor so marked as in the arteries.

Both the arteries and veins of the control animals were normal in every respect, as were those from the animals killed within eight days after first receiving irradiated ergosterol.

3. *Tubules:* Extensive pathological changes occurred in the renal tubules of all animals that received irradiated ergosterol. Structural changes were much more apparent in the convoluted tubules and the loops of Henle than in the collecting tubules. The most conspicuously involved tubules were in the vicinity of the clusters of affected vessels described above. In the severely damaged kidneys there were focal areas in which the tubules showed marked calcification and hyalinization and thickening of the basement membrane. In a few of the most severely damaged kidneys, the majority of the tubules were markedly atrophic. In the less damaged kidneys the majority of the tubules were undergoing cystic dilatation, with little or no thickening of the basement membrane. There were many areas containing tubules in various stages of transition

arteries was rarely involved. In both the arterioles and the interlobular arteries showing advanced changes, the walls were thickened with resulting diminution in the caliber of the lumina. Frequently the contour of the vessels was altered and the lumina were eccentrically placed. Many of the lumina were reduced to mere slits, or even entirely obliterated. In such cases the sites of former lumina were occupied by fused masses of necrotic material, calcium salts and cellular débris. In some instances polymorphonuclear and endothelial leucocytes were found in this material.

*Calcium and Hyalin:* Large and small irregularly shaped masses of calcium were deposited in the walls of the interlobular arteries. In the arterioles the deposits were smaller and less numerous. The calcium deposits in the arteries were located chiefly in the media and internal elastic lamina. In some vessels these deposits in the media were sharply separated from one another by normal tissue, while in others they fused to form a calcified ring encircling the lumina. These deposits occurred both in normal appearing and necrotic areas of the vessel walls. After decalcification many of the areas formerly occupied by calcium appeared either as hyalinized material or as degenerated regions containing vacuolated cells without nuclei or with pyknotic nuclei.

In many instances the calcification and hyaline formation were so intimately related that it was impossible to differentiate sharply between them. At times, however, a definite layer of hyaline material lay internal to the calcium deposits. After removal of the calcium the bluish pink-staining hyaline material became more prominent. It was deposited in areas of irregular size and shape in the media and subintimal portion of the interlobular arteries, and as a subendothelial band of irregular thickness and distribution in the glomerular arterioles. This irregular distribution was especially well shown in longitudinal sections of the affected vessels. The tissues immediately adjacent to the hyaline deposits were well demarcated from them and appeared normal. Instances were observed in which the afferent arteriole was normal, but the interlobular artery leading to it was undergoing hyalinization. Areas of necrosis were occasionally found in the hyalinized regions. In general the amount of hyalinization paralleled the degree of vessel involvement.

*Fat:* Many of the severely damaged vessels were heavily laden with droplets of fat. These deposits were larger and more numerous



basement membrane. They either took the form of isolated deposits of variable size separated by normal appearing tissue, or fused to form crescentic masses. After removal of calcium there remained either areas of hyalinization or regions of vacuolization containing pyknotic nuclei and cellular débris. An irregularly thickened bluish pink layer of hyaline material lay between the basement membrane and the epithelium. This hyaline band sometimes extended around the capsule and became continuous with the hyaline layer of the tubule. At times the glomerular capsule was somewhat shrunk. In the capsules damaged to the greatest extent there were considerable deposits of fat, while scanty deposits were found in the capsules only moderately injured. A few of the tufts contained focal deposits of calcium but the great majority were normal. There did not seem to be a close relationship between the amount of blood in the glomerular tufts and the degree of vessel damage.

The glomeruli in the kidneys of the control animals and those killed early were normal.

The interstitial tissue was essentially negative in the kidneys of all animals.

#### CHEMICAL CONSTITUENTS OF THE BLOOD

The effect of the administration of irradiated ergosterol upon the nitrogenous constituents of the blood can best be appreciated by considering first the concentration of these substances in the blood of the control rabbits. As shown in Table II, the non-protein nitrogen averaged 39 mg. per 100 cc., only two blood samples showing more than 43 mg. per 100 cc. The urea nitrogen averaged 15 mg. per 100 cc., creatinine 1.6 mg., and uric acid 1 mg. The value for uric acid was about one quarter as great as is normal for human blood.

The bloods of the animals receiving fatal doses of irradiated ergosterol all showed a rise in the nitrogenous constituents during the last day or two of life, the terminal values for non-protein nitrogen ranging from 67 mg. to 222 mg. per 100 cc., as shown in Table I. The urea showed a comparable but proportionately greater rise, so that at death the urea nitrogen amounted to about 65 per cent of the non-protein nitrogen, whereas in the normal animals it was only about 35 per cent. This same phenomenon occurs commonly in human beings, as a result of nephritis.<sup>11</sup> The blood creatinine in-

between simple cystic dilatation and the more advanced condition of calcification and thickening of the basement membrane. Calcium was deposited in and near the basement membrane of many of the severely damaged tubules, and to some extent within the degenerated epithelium. The calcium appeared as large or small isolated masses of irregular contour which often fused to encircle the lumina. The transition was abrupt between areas of calcification and immediately adjacent normal tissue. Frequently large, laminated, often oval-shaped masses of calcified and hyalinized material occurred within the thickened basement membrane. These masses indented the tubular walls and many times invaginated the walls into the lumina. A thickened, coarse, irregular zone of almost transparent bluish pink, hyalinized material often lay between the epithelium and the basement membrane. At times this layer was as thick as the original diameter of the tubular epithelium. Where the calcium deposits and hyaline changes were most marked the tubules became narrowed, distorted, and at times difficult to identify. In the severely damaged tubules the epithelium usually was degenerated, but in some of them it remained practically normal. The early changes in the epithelium consisted in hyaline droplet degeneration with scattered droplets of fat, or else swelling of the cells resulting in pronounced narrowing or even occlusion of the lumina. In the animals living the longest, however, the cells were markedly flattened and contained relatively few fat droplets. The tubules were often distended with large hyaline casts containing calcium.

The collecting tubules showed to a much less degree the changes described in the convoluted tubules and in Henle's loops. However, the most noticeable abnormality was the presence of large hyaline casts containing calcium. They distended the tubules and compressed the lining epithelium.

The tubules in the kidneys of the control animals were normal, as were those of the animals killed soon after the administration of irradiated ergosterol was begun.

4. *Glomeruli*: In the severely damaged kidneys the glomerular capsules were often thickened, hyalinized and calcified. In the kidneys injured but moderately or slightly, the capsules almost always appeared normal. The pronounced lesions in the glomerular capsules were located near groups of severely damaged vessels and tubules. The calcium occurred in irregular deposits in and near the

TABLE I  
Effect of Massive Doses of Irradiated Ergosterol ("Super-Acetrol") upon Rabbits

Exp. No.	Duration	Total dose	Number of doses	Day of experiment	Weight of animal	Albumin in urine	Hemo- globin	Blood				Degree of calcification of kidney vessels
								Non-protein nitrogen	Urea nitrogen	Creatinine	Uric acid	
	days	cc.			gm.		per cent	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	
40	60	106	12	1st	2850	0	70	67	38	2.8	1.6	
				43d	2680	0	70	48	27	1.8	0.9	
				59th	2400	..	65	120	100	2.5	2.0	
				60th	2250	..	65	222	163	2.8	2.0	++++
37	13	40	4	1st	1830	0	65	44	17	2.1	0.7	
				13th	1320	++++	..	169	115	4.5	1.3	++++
16	14	97	14	1st	2080	0	60	36	16	1.7	0.9	
				3d	1980	0	60	20	14	1.2	1.3	
				7th	1920	0	60	38	13	1.8	1.0	
				10th	1790	0	60	38	17	1.7	0.7	
33	19	71	8	14th	1680	++++	60	160	111	5.2	1.7	++++
				1st	2765	0	60	53	20	3.3	1.1	
				14th	2200	++++	70	61	32	3.0	1.3	
				16th	1980	++++	..	65	38	4.2	1.6	
34	14	69	9	19th	..	..	..	156	115	4.0	1.5	++++
				1st	2890	0	70	52	26	1.6	3.2	
				14th	2340	..	..	154	95	4.5	4.9	++++
				1st	2480	0	70	60	25	3.2	0.8	
39	11	30	3	11th	2020	++++	65	115	76	5.5	1.4	
31	15	66	15	1st	2715	0	70	36	12	1.7	0.6	
				11th	2105	..	70	52	28	2.9	0.5	
				15th	1980	++++	70	80	43	2.2	0.7	
				1st	1920	0	70	42	17	1.7	0.5	+++
29	12	57	12	11th	1675	++++	65	76	46	3.0	1.0	++++
20	9	85	9	1st	1830	0	60	48	15	1.8	0.5	
				3d	1760	0	60	31	15	1.2	0.8	
				6th	1620	0	55	40	15	1.8	0.4	
				9th	1460	0	50	75	42	2.9	0.8	+
30	12	55	12	1st	1920	0	55	37	20	1.7	0.5	
				12th	1440	++++	..	67	32	2.8	0.8	++
				Average at death .....				127	84	3.7	1.6	

creased to a rather less degree than the non-protein nitrogen and urea, while the uric acid never rose to more than twice its original level and in several instances showed scarcely any rise at all. This last finding is in accord with Folin's observation<sup>12</sup> that herbivorous animals excrete uric acid with extraordinary efficiency.

The kidneys of some of the rabbits showed much more marked calcification than those of others, and in turn it is evident from the data given in Table I that the height of the non-protein nitrogen at death corresponded quite closely with the degree of calcification and general kidney damage. It is probable that the animals which showed relatively less kidney involvement *post mortem* died on account of lesions in other organs, while in those showing the most marked involvement of the kidneys, uremia was at least an important contributory cause of death. It is noteworthy that in all cases the retention of nitrogenous products occurred only in the last few days of life, after the kidney damage had become severe.

In order to determine the effect of extreme cachexia upon the chemical constituents of the blood discussed above, two rabbits in the last stages of tuberculosis were studied. The nitrogenous constituents were not found to be elevated.

## DISCUSSION

It has been shown in the experiments reported here that excessive doses of activated ergosterol administered over a period of from nine to sixty days produced marked histological changes in the renal vessels, tubules and glomerular capsules. The media of the interlobular arteries and the entire walls of the glomerular arterioles were often sclerosed, and both types of vessels contained prominent subendothelial deposits of hyaline material. In addition the internal elastic lamina of the interlobular arteries was thickened, and interweaving and splitting of the fibers occurred. The basement membrane of the tubules was markedly thickened and large areas of hyalin lay between it and the tubular epithelium. In some animals marked tubular atrophy occurred. The basement membrane of the glomerular capsules was likewise irregularly thickened, with large deposits of hyaline material between it and the capsular epithelium. Calcification was marked in isolated areas in and near the basement membrane of the tubules and of the glomerular capsules. The same

condition also occurred in the vicinity of the internal elastic lamina of affected vessels.

This process was accompanied by a retention of nitrogenous products in the blood and the appearance of albumin in the urine, indicating serious interference with renal function.

The composite histological changes in the kidneys of the animals were strikingly different from the picture of any known pathological process occurring in man. Nevertheless, after decalcification, the lesions in the glomerular arterioles and the interlobular arteries did resemble the renal lesions associated with hypertension. Blood pressure readings were not taken because of technical difficulties.

Another unusual feature was the frequent occurrence of widespread tubular atrophy with very little demonstrable damage to the glomerular tufts. It is thought that perhaps this atrophy was secondary to vessel damage and to the tremendous thickening of the basement membranes of the tubules.

Collip *et al.*<sup>13</sup> has reported a rise in the non-protein nitrogen of the blood of dogs to as high as 219 mg. per 100 cc., occurring just before death as a result of large doses of parathyroid hormone. This substance, like irradiated ergosterol, causes a marked increase in blood calcium, with calcium deposition in various organs. Kidney lesions occur, as reported by Hueper<sup>14</sup> and Learner,<sup>15</sup> but the parenchyma is more seriously affected than are the blood vessels. Hueper describes, in dogs that received large doses of parathyroid extract, hemorrhages in the glomeruli, necrosis and calcification of the tubular epithelium, and "calcification of the membranæ propriae of the tubules and Bowman's capsules and of the elastic membranes of the arteries." Learner records similar changes in rabbits, but states that "it was in the lumen of the tubuli where the calcium was most to be seen."

It is noteworthy that the doses of irradiated ergosterol used in our experiments were many times greater, per unit of body weight, than those recommended for human use.

## SUMMARY

1. The administration of massive doses of irradiated ergosterol to rabbits caused marked histological changes in the kidneys. The chief changes were sclerosis and hyalinization of the vessel walls, and thickening of the basement membranes of the tubules and

TABLE II  
*Effect of Inert Solvent Oil upon Rabbits*

Exp. No.	Duration	Total dose	Day of experiment	Weight of animal	Albumin in urine	Hemo- globin	Blood				Description of kidneys
							Non-protein nitrogen	Urea nitrogen	Creatinine	Uric acid	
28	days 44	cc. 325	1st	gm. 2360	..	..	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	Normal
			14th	2170	o	70	30	14	1.7	0.8	
			28th	1950	o	65	39	18	3.0	1.0	
			44th	1820	o	65	36	15	2.1	0.5	
19	33	250	1st	2810	o	60	38	11	1.8	2.0	Normal
			4th	2770	o	60	35	9	1.7	1.6	
			7th	2610	o	60	43	17	1.7	1.6	
			31st	2440	o	60	40	18	2.3	1.6	
42	21	210	1st	2050	o	70	43	9	1.5	0.8	Normal
			21st	2120	+	70	34	9	1.5	1.0	
43	21	210	1st	2350	o	70	32	13	0.9	1.0	Normal
			21st	2400	o	70	32	11	1.5	0.6	
44	21	210	1st	2450	o	65	37	18	1.1	0.8	Normal
			21st	2500	++	65	32	11	1.5	0.8	
45	21	210	1st	2150	o	60	39	20	1.3	1.1	Normal
			21st	2180	o	70	34	10	1.2	1.5	
21	18	95	1st	1740	o	55	43	18	1.8	0.9	Normal
			3d	1670	o	55	40	17	2.1	0.9	
			6th	1580	o	55	55	21	1.5	1.0	
			10th	1530	o	50	34	11	1.2	0.7	
			13th	1470	o	50	29	14	1.4	0.5	
			18th	1240	..	..	40	23	1.4	0.9	
17	11	75	1st	2450	++	60	32	7	1.3	0.5	Normal
			3d	2400	o	60	35	9	1.6	0.6	
			8th	1900	o	60	75	46	1.7	0.5	
Average.....							39	15	1.6	1.0	

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## DESCRIPTION OF PLATES

### PLATE 101

- FIG. 1. Shows numerous dark staining deposits of calcium in the cortex and deep in the medulla.  $\times 15$ .
- FIG. 2. Four arterioles undergoing hyalinization, and narrowing. Also note hyaline deposit in a portion of the glomerular capsule.  $\times 500$ .

glomerular capsules, accompanied in both by extensive subepithelial deposits of hyalin. There was abundant deposition of calcium in these localities. Pronounced atrophy of the tubular epithelium also occurred.

2. The kidney lesions were accompanied by the appearance of large amounts of albumin in the urine and by retention of nitrogenous products in the blood. The degree of nitrogen retention was in general proportional to the amount of kidney damage as evidenced by histological examination.

We wish to acknowledge our indebtedness to Dr. F. B. Mallory for helpful criticism and for the photomicrographs.

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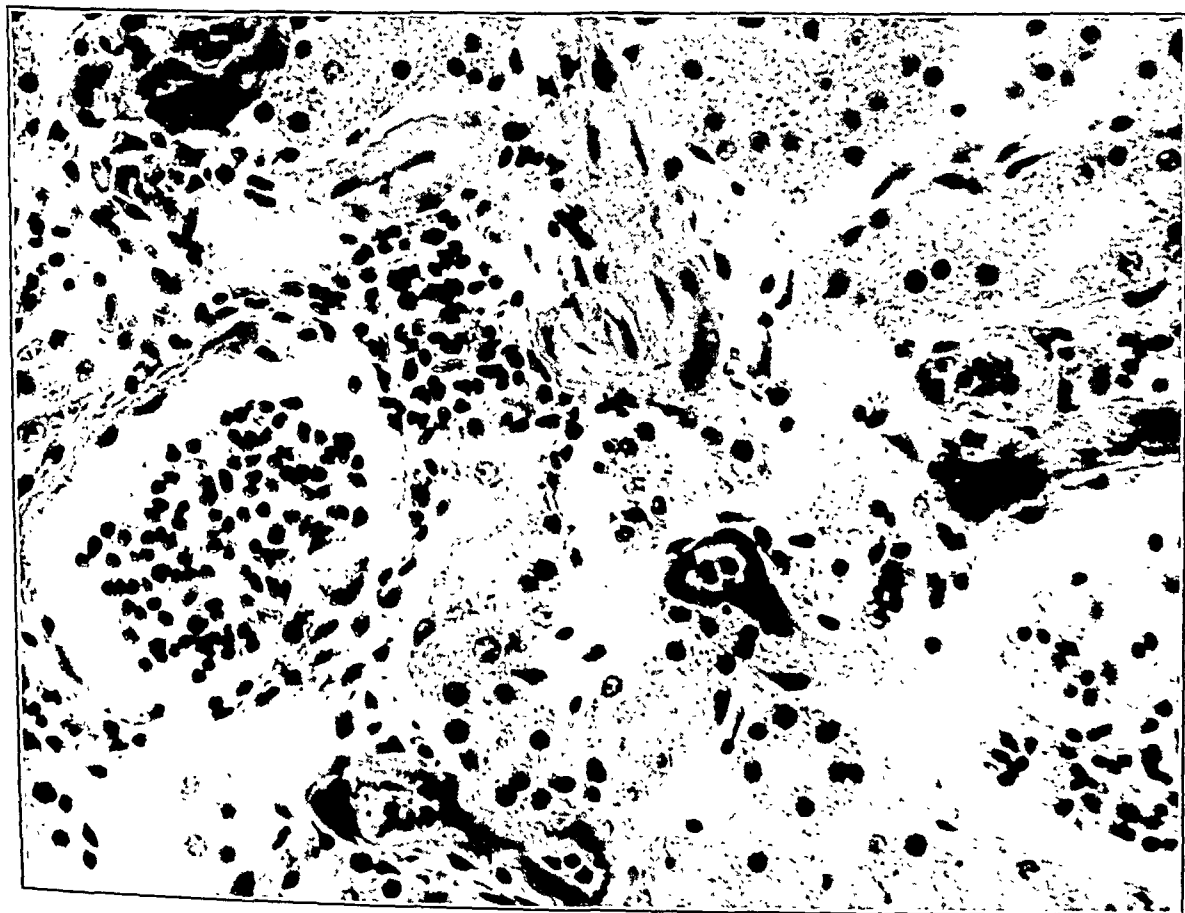


PLATE 102

- FIG. 3. Large interlobular artery with irregularly distributed subintimal deposits of hyalin. Adjacent arteriole, shown in longitudinal section, is undergoing marked hyalinization with reduction in caliber.  $\times 500$ .
- FIG. 4. Focal area of atrophied tubules containing thick deposits of hyalin. Note a portion of large artery is essentially negative. Compare these tubules with the more normal tubules in Fig. 2.  $\times 500$ .
- FIG. 5. Marked atrophy of the tubules with resulting approximation of the three glomeruli. Compare these atrophic tubules with the more normal tubules in Fig. 2.  $\times 500$ .



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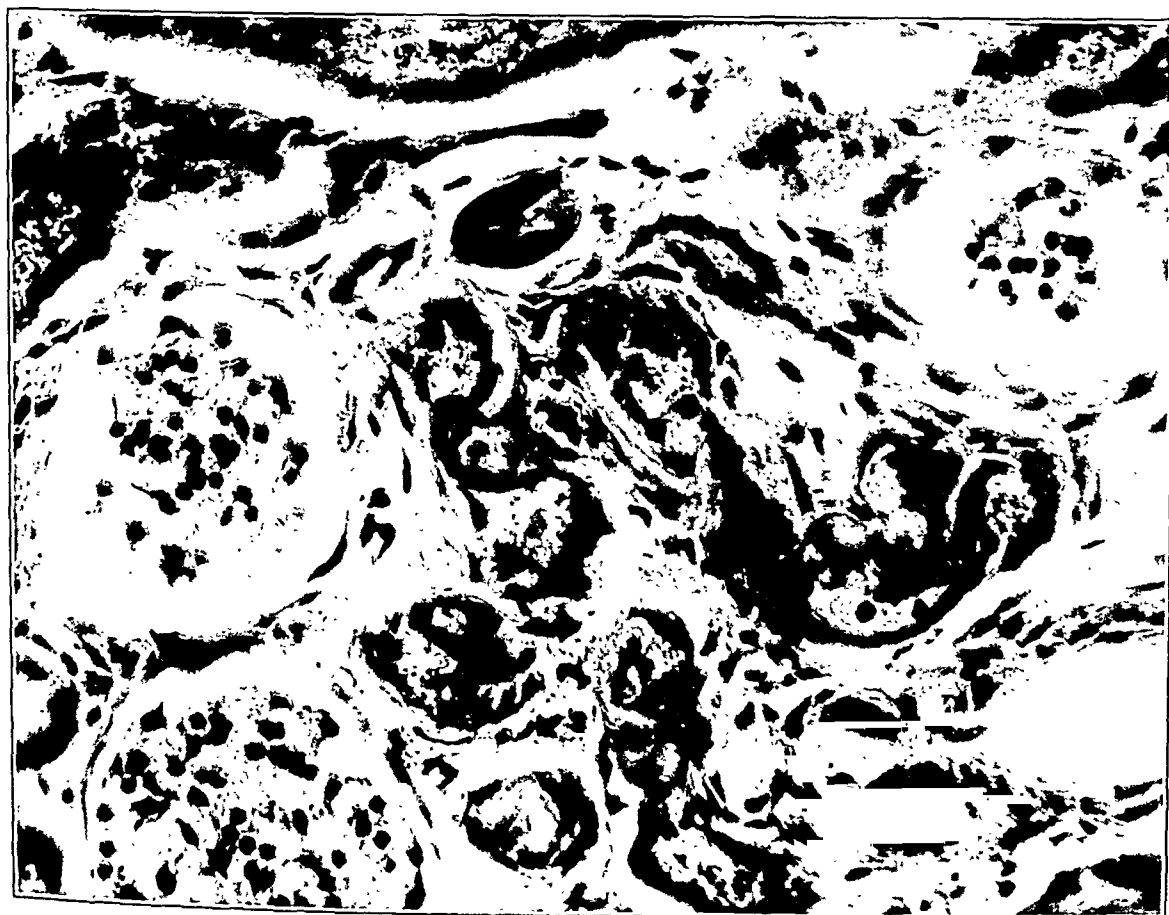




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## THE SIGNIFICANCE OF THE MUSCULAR "STROMA" OF ARGENTAFFIN TUMORS (CARCINOIDS) \*

P. MASSON

*(From the Department of Pathological Anatomy, University of Montreal,  
Montreal, Canada)*

The physiological rôle of the argentaffin cells remains absolutely unknown. From the beginning of my researches I had the idea, since expressed by Danisch, of using extracts of carcinoids in physiological experiments. Circumstances hitherto have not favored the realization of this project. In the meantime, may not a purely morphological study of carcinoids point the way.

We have long known that most carcinoids possess a stroma more or less rich in smooth muscle fibers. In his remarkable chapter devoted to these tumors, Oberndorfer<sup>1</sup> hesitates over the interpretation of these fibers. For him, it is not improbable that they come from the muscularis mucosae. "They have no connection whatever with the circular and longitudinal muscle coats and, for the opinion that they form an integral part of the tumor, there is much to be said (vieles für sich hat)." Comparison of several carcinoids of different degrees of development and of invasion will lead us to a more exact and perhaps more suggestive interpretation.

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### OBSERVATIONS

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### CORRECTION

PLATE 85. Fig. 14 should have legend of Fig. 15.

PLATE 86. Fig. 15 should have legend of Fig. 16. Fig. 16 should have legend of Fig. 14.

gravid uterus. Submucosa fibrous, traversed by voluminous muscle bundles that unite the circular muscle and the muscularis mucosae. Nerves and ganglia of Meissner's plexus hypertrophied. Muscularis mucosae notably hypertrophied.

The thick mucosa poor in lymph nodules. Crypts broad, elongated, the cells hypertrophied, most of them containing mucus in

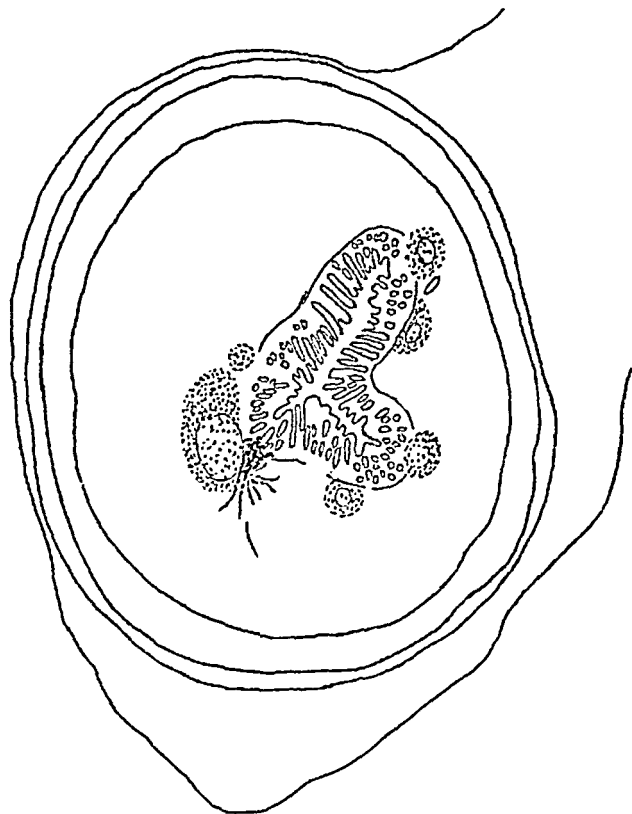


DIAGRAM I

large calices. The stroma of the mucosa consists of the usual reticulum, and especially of a network of slender nerve fibers deprived of argentaffin cells. The lymph filling the meshes of this reticulum is poor in free cells.

Two areas, the tip and the middle of the appendix, present special features. At the tip of the appendix there is a voluminous argentaffin cell neuroma. This neuroma is so large that it has ruptured the muscularis mucosae and the muscle coats. It is capped by an attenuated submucosa which makes a hernia beneath the visceral peritoneum.

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### OBSERVATIONS

*Obs. 1 (93 c):* Diagram 1. Voluminous appendix with thickened walls. The thickening affects the submucosa almost exclusively.

In its entire length the appendix presents the lesions characteristic of hypertrophy of the musclonervous complex of the submucosa (Masson<sup>2</sup>). Fibers of the muscle coat voluminous, like those of a

\* Received for publication April 30, 1930.



Throughout the appendix the subglandular plexus is moderately hypertrophied. Here and there are beginning neuromas. The nerve filaments contain round argentaffin cells, either single or in small groups, always few in number.

The submucosa is normal in thickness. The nerves of Meissner's plexus are voluminous and the number of muscle bundles is notably increased. There is nothing abnormal in Auerbach's plexus or in the



DIAGRAM 2

muscle coats. There is, then, both hyperplasia of the nerves of the mucosa containing argentaffin cells, and moderate hyperplasia of the musclonervous complexes of the submucosa.

Near the tip of the appendix, embedded in the submucosa and occupying its inner two-thirds, is a small sphere colored red by the trichrome stain. This minute tumor is continuous with the muscularis mucosae but it does not reach the circular muscle. Only a few slender bundles detach themselves from the circular muscle, as in all appendices, and fuse with the outer surface of the tumor. Examina-

In the middle of the appendix, between the tips of three or four crypts and the slightly bulging muscularis mucosae, is a group of typical carcinoid columns. These columns are slender; they consist of polygonal and especially of palisaded cells. The cytoplasm of these cells contains fine acidophilic granules gathered at one pole. They do not reduce silver.\* Some of them have two nuclei, probably an indication of amitotic division.

The carcinoid columns anastomose in a plexus. They are enclosed in a thin sheath of collagen. It can be seen easily that they are not situated in the lymphatic interstices of the mucosa but in the nerves themselves; they have penetrated the nerves and adopted their plexiform structure. Alongside of the area that contains the carcinoid cells, the nerves are slightly hypertrophied and pressed more closely together than in the rest of the appendix.

These columns occupy a very restricted area of the mucosa; some of them send prolongations into the nerves of the muscularis mucosae; they are found even as far as the superficial filaments of the submucous plexus. It is obvious that the carcinoid, intranervous in its entire extent, has arisen in the subglandular nerves and that the invasion of the nerves of the muscularis mucosae and of the submucosa is comparatively recent.

The contractile cells of this region are slightly hypertrophied but in this they do not differ noticeably from those of the other regions of the appendix. It is obvious that they were there before the appearance of the tumor and that they do not form an integral part of it in any sense.

*Obs. 2 (241 h):* Diagram 2. Appendix with uniform lumen. Cavity filled with pus.

The mucosa, rich in lymph nodules, five to seven per cross-section, presents no ulceration. Discrete infiltration with polynuclears in its superficial region. Deeper down, as in the submucosa and the muscle coats, there are no wandering cells. No peritoneal reaction. This appendix, then, was removed during a simple catarrhal inflammation which has not altered its structure in any way.

\* I have stated elsewhere that the silver-reducing properties correspond to a functional state of the argentaffin cells; that the silver-reducing granules are not found in all of the cells of carcinoids; and that some of these tumors are completely free from them, although their nature cannot be doubted for all that. In the absence of these granules the carcinoid cells have enough other special characteristics to make their identification easy.

In the mucosa and capping the tips of three crypts of Lieberkühn, slender carcinoid columns form an almost continuous plexus and penetrate a tiny intramucous neuroma. Outside of the invaded areas, the nerves are intact and have not undergone any degeneration, either above or below the penetrated points or between them. The adjacent portion of the muscularis mucosae is much thickened and forms a small fasciculated myoma which encroaches on half the thickness of the submucosa.

Laterally, this myoma of the muscularis mucosae anastomoses with another myoma occupying the outer half of the submucosa and

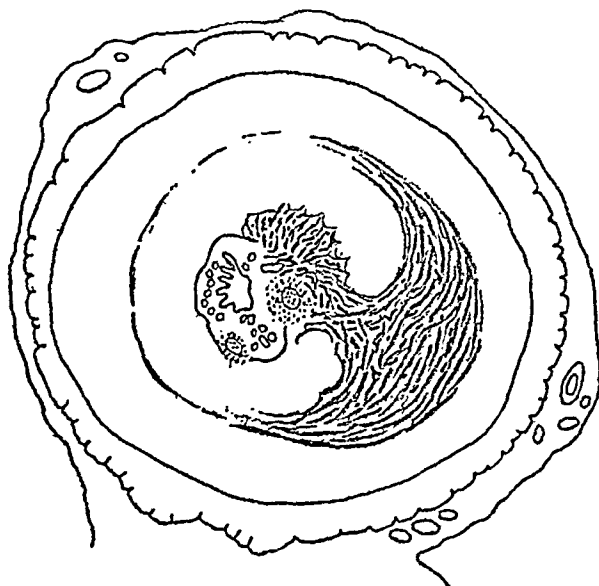


DIAGRAM 3

attached externally to the circular muscle. This latter myoma, conical in form, has a truncated summit by which it is continuous with the myoma of the muscularis mucosae; it seems to represent an intense hyperplasia of some of the muscle bundles of the submucosa. Its bundles are formed of large fibers rich in myofibrils. Its broad base corresponds to the middle zone of the circular muscle. In all of this external portion the contractile fibers are poorly differentiated, with few myofibrils, clear and oriented in all directions without appreciable bundle formation.

It is obvious that this myoma results from hyperplasia of preëxisting muscle fibers and not from the production of entirely new muscle fibers between the old bundles. If the latter condition obtained,

tion with high magnification shows this tumor to be a myoma with cells elongated and running in all directions. Its interstices are occupied by collagen fibers, many capillaries, slender nerve filaments, and small clusters of argentaffin cells.

Between the myoma and the tips of the crypts of Lieberkühn is found a hypertrophied nerve plexus rich in argentaffin cells. This plexus is continuous with the rich and complex nerve network which ramifies among the fibers of the adjacent myoma. Without any possible doubt, the myoma is the result of hyperplasia of the muscularis mucosae.

The carcinoid columns included in this neuroma do not infiltrate the connective tissue, but they penetrate the nerve filaments and assume their plexiform arrangement. This penetration is not general. At certain points where the carcinoid columns are interrupted, they are seen to be continued by nerves which are intact but more slender, and it can be seen also that the carcinoid columns are separated from the surrounding tissue by a thin sheath of collagen which is continuous with that of the still uninvaded nerve.

Without doubt, this carcinoid arose in the nerves of the mucosa. Its proliferating cells invaded the nerves of the muscularis mucosae and the contractile fibers of this muscle have multiplied, giving rise to a myoma which, from the mere fact of its origin, occupies the interstices of the invaded nerve plexus.

*Obs. 3 (123 m):* Diagram 3. Appendicular lumen regular but narrow and cylindrical. Lymphatic tissue quite abundant, five to ten nodules in each cross-section.

The subglandular nerve plexus contains a number of isolated argentaffin cells. This plexus is hyperplastic; here and there it forms tiny neuromas which push the muscularis mucosae outward. The submucosa is adipose; it presents muscle fibers and nerves that are moderately but distinctly hyperplastic. In short, along its whole length this appendix shows typical hyperplasia of the subglandular plexus containing many argentaffin cells, and hyperplasia of the musclonervous complex of the submucosa.

Near the tip there are special lesions which merit closer attention. Around four-fifths of its circumference the appendix has the characteristics just described, except that it is poorer in lymph nodules, two to each cross-section. The remaining fifth presents the following peculiarities.

dix. Many, probably all of them, are intranervous. In all this region this coat is much thickened, whereas it is very thin on the opposite side where there are no argentaffin cells. It should be noted that at one point (upper part of the figure), the new fibers issuing from the external layer of the longitudinal coat pursue a very abnormal course; most of them are circular.

Having traversed the muscle coat, the tumor columns invade the serosa where they acquire a much larger caliber. In all of this outer zone they are not in the nerves but in a connective tissue normally deprived of muscle fibers. This tissue becomes denser between the

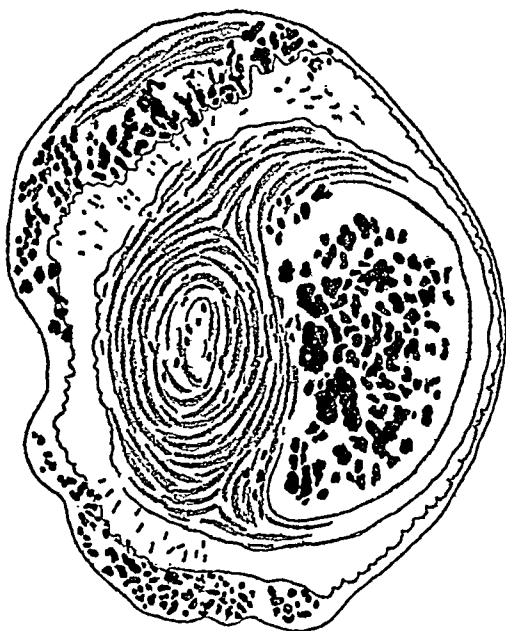


DIAGRAM 4

columns but there is not a single muscle fiber in the interstices. At this point, the stroma of the carcinoid contains no smooth muscle fibers.

*Obs. 5 (Mag.):* Diagram 5. Last centimeter of the appendix obliterated, occupied entirely by a carcinoid.

The tumor fills the axis and penetrates the muscle coat at a narrow point. From this point, the carcinoid columns spread through the interstices of the circular muscle around three-fourths of its circumference, leaving one-fourth free. Finally they gain the longitudinal muscle where they spread radially.

The stroma of the axial portion of the tumor is very fibrous but it

adult circular fibers would be found among the disoriented young fibers. Now, these latter alone are present to the exclusion of the others throughout the whole myoma which occupies the place of the circular muscle. Moreover, this area of young fibers is continuous externally by all possible transitions with the normal muscularis of adult circular fibers.

Summing up these various myomas, we find that each of them has for its origin a special muscle apparatus, that is, muscularis mucosae, muscle complex of the submucosa and circular muscle. We learn further that their muscle fibers are well differentiated and at rest in the myomas nearest the mucosa, young and in active proliferation in the myoma of the circular muscle. The latter is obviously more recent than the others.

The interstices of all these myomas are invaded by the carcinoid, the *neurocarcinoid*, originating in the mucosa, and here too the invasion is not into the connective tissue but purely nervous. The invasion is abundant enough to mark out the reticular structure of the affected plexus, discrete enough to permit recognition of the segments of intact nerves between the infiltrated portions, and so distinct that its limits can be determined precisely. The muscular hyperplasia is produced exclusively at the expense of the muscle coats, the nerves of which have been infiltrated; in the interstices of the mucous portion of the carcinoid there are no muscle fibers. It is most mature in the earliest invaded areas; for instance, in the muscularis mucosae it consists of adult muscle fibers, in the circular muscle of young fibers.

*Obs. 4 (320 h):* Diagram 4. Carcinoid of the tip of the appendix.

The carcinoid is separated from the end of the appendicular lumen by a voluminous, axial, argentaffin cell neuroma in which, beyond any doubt, it has arisen (*neurocarcinoid*). The tumor fills the appendicular axis and penetrates the circular muscle at a very restricted point, invading only the myenteric nerves and this in a very discrete manner. All of that region of the circular muscle around the infested nerves has undergone considerable hyperplasia, resulting in a lenticular-shaped myoma. This myoma, at its margin, is continuous with the circular muscle; it encroaches on and deforms the carcinoid.

Slender argentaffin columns gain the longitudinal muscle and cross it here and there around one-half of the circumference of the appen-

The tumor is an enormous myoma with very compact muscle fibers. There are three regions differing in the orientation of their fibers; in the middle the fibers are longitudinal; at the periphery they are circular; in the intermediate zone they are oblique. Judging from its situation within the submucosa, this myoma manifestly corresponds to an enormous hyperplasia of the muscularis mucosae. Its interstices are infiltrated by many carcinoid columns, most of them intranervous.

If we turn now to the muscular coats of the appendix, we find that for four-fifths of their circumference they are very thin and sepa-



DIAGRAM 6

rated from the central myocarcinoid by the submucosa. In the remaining fifth (left side of the figure) they are thick and the circular muscle is connected with the myoma by anastomotic fibers. In this region, and in this region only, Auerbach's plexus is infiltrated with carcinoid columns. Some of these columns pass beyond the plexus and the longitudinal muscle to invade the connective tissue of the visceral serosa. The stroma of this peritoneal portion of the tumor does not contain a single muscle cell.

*Obs. 7 (102 f):* Diagram 7. (In this diagram, the contour of the axial carcinoid is indicated by a festooned line.)

is easy to distinguish many smooth muscle fibers continuous with the vestiges of the muscularis mucosae.

In all of the muscle region invaded, nearly all, perhaps all, of the carcinoid cells are in the nerves and each of these coats presents notable thickening, maximum at the point of penetration of the circular muscle by the carcinoid, diminishing gradually at each side in the regions more recently invaded, to cease beyond the zone of invasion.

It should be noted further that a group of argentaffin columns have penetrated the nerves and perhaps the lymphatics which run

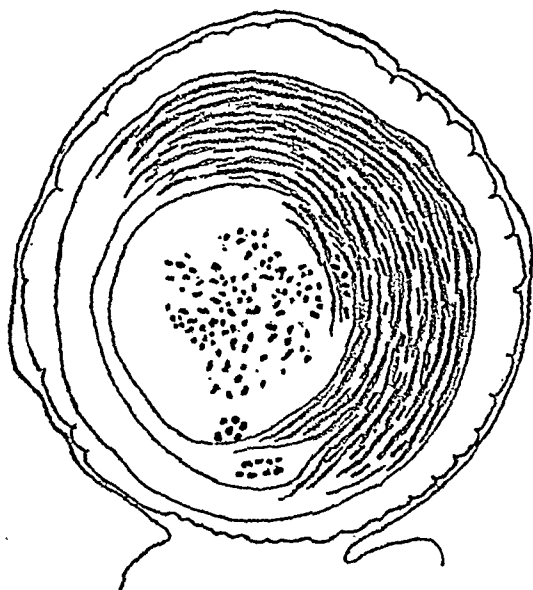


DIAGRAM 5

alongside of a large artery coming from the meso-appendix. These columns traverse the muscle coats without putting out prolongations into their nerves. Despite this propinquity, the muscle fibers do not undergo any change either of dimensions or number.

*Obs. 6 (31 m):* Diagram 6. Carcinoid at the tip of the appendix.

The nearly spherical tumor measures about 7 mm. in diameter. It is separated from the circular and longitudinal muscle by a thin submucosa. Excentrically placed, there is a small rounded group of voluminous carcinoid columns separated by purely connective tissue septa. This tiny object probably represents the axis of the obliterated appendix, the point of origin of the carcinoid, pushed to one side by the unequal growth of the rest of the tumor.



muscle sheath, representing the muscularis mucosae, separates them. At one point only, the muscularis mucosae is traversed by carcinoid columns which penetrate the muscle coats along one-fifth of their circumference and gain the serosa. Attentive study of this zone of invasion shows that many of the carcinoid columns are not really in the interfascicular connective tissue but in the filaments of Auerbach's plexus.

In all of the invaded region the circular and the longitudinal muscle coats are thickened. The thickening doubtless depends in part on the infiltration of their interstices with foreign cells, but chiefly on the multiplication and hypertrophy of the contractile cells themselves. A little beyond the zone of invasion the thickening ceases rapidly.

We should note an important point. The carcinoid columns traverse the muscle coats, either through the nerves or through the interstitial tissue, to infiltrate the peritoneal nerve filaments and connective tissue. In these regions where there is no preëxistent muscle, the stroma of the tumor is pure connective tissue; but if, as in the section represented in the figure, the argentaffin cells following the nerves gain a muscle bundle belonging to the meso-appendix, this muscle bundle undergoes local hypertrophy identical with that of the appendicular muscle.

## DISCUSSION

(a) The small carcinoids which I have been able to observe in permeable appendices begin in tiny neuromas situated in the subglandular portion of the plexus of the mucosa. Several observations related in this paper, as well as others not reported here, lead me to believe that when carcinoids develop in previously obliterated appendices, they arise in axial neuromas resulting from the persistence or increase of the tiny neuromas already mentioned. All of the young carcinoids that I have seen are *neurocarcinoids*.

All of the proliferating argentaffin cells are of the glandular type. They penetrate the nerves electively without provoking either their increase (this was acquired previously) or their degeneration; therefore the cells are not neurogenic. If, then, the initial neuroma has been provoked by certain argentaffin cells, it is not by these glandular cells, for their multiplication has not this effect, but by others

An appendix 6 cm. long, regularly cylindrical, except at the tip which is slightly swollen; at this point, the peritoneum is yellow and opaque, foretelling the presence of a carcinoid. In the first 3 cm. the appendix is permeable; in the last 3 cm. obliterated.

The permeable region need detain us little. In the mucosa, lymph nodules are few; between them, the subglandular plexus is slightly hyperplastic and contains a few argentaffin cells. There is no ab-

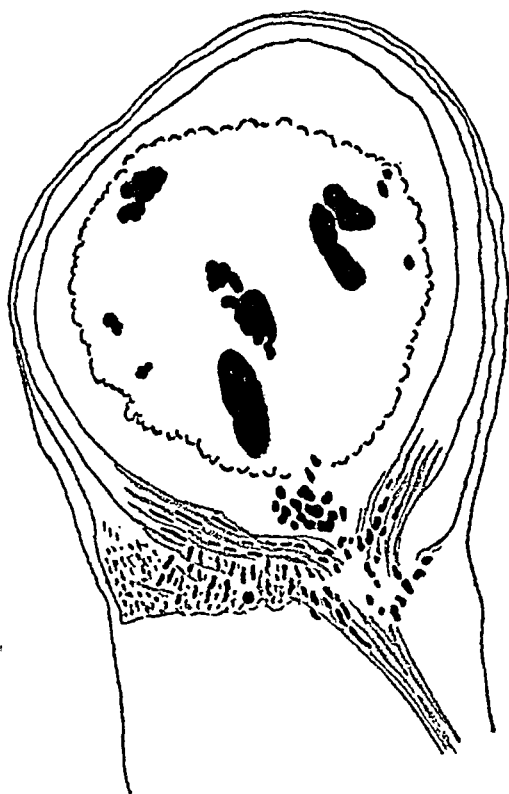


DIAGRAM 7

normality in the musculonervous complex of the submucosa nor in the muscle coats.

The distal four-fifths of the obliterated region is occupied by a carcinoid, the proximal fifth by a voluminous argentaffin cell axial neuroma, continuous with the plexus of the mucosa. The carcinoid invades the neuroma from below upward and we may suppose that it originated in the terminal region of this neuroma, which it replaces (neurocarcinoid).

The carcinoid fills the axis of the appendix but does not invade the submucosa, which is thinned and pushed before it. A discontinuous

every muscle region where the nerves are infested with argentaffin cells.

(c) What might be the cause of this muscular hypergenesis? Elsewhere<sup>2</sup> I have shown that the appendicular muscles, those of the muscularis mucosae, those of the submucosa and those of the muscle coats, may undergo a hyperplasia, the cause of which seems to be hyperplasia of the myenteric sympathetic. In the lesion under discussion, it is probable that here again the muscle hyperplasia is due to a nervous excitation but of quite another nature; it seems to be determined by the carcinoid invasion of the same plexuses.

One might think that the mere presence of foreign cells in the nerve filaments would excite them and provoke hyperplasia of the corresponding muscle territories. Were this true, invasion of the myenteric plexus by ordinary cancer cells should have a similar effect; but nothing like this is seen. May we not suppose, then, that the muscle hyperplasia is incited by a special intranervous secretion, a *neurocrinia*<sup>3</sup> of the carcinoid cells?

What is this substance secreted by the argentaffin cells? In these cells are found two kinds of inclusions, fats and granules. The fats are complex; their microchemical characters show that they consist of a mixture of neutral fats and cholesterin ethers in variable proportions. In argentaffin cell neuromas I have observed that these fats elaborated in the cells may be eliminated directly into the nerve filaments where they soon disappear, as if they had been utilized by the nerve tissue. The argentaffin granules are as chromaffin as are those of the paraganglia, hence the idea proposed by Ehrlich,<sup>4</sup> by myself,<sup>5</sup> and subsequently by Danisch,<sup>6</sup> that the carcinoids are phaeochromoblastomas or paragangliomas comparable to the tumors of the adrenal medulla.

Ehrlich and Danisch believed that the carcinoids were sympathetic phaeochromoblastomas analogous to those of the adrenal. The certain endodermic origin of the carcinoid cells, their argentaffinity together with their chromaffinity, have led me to an opinion which is at the same time similar and different. For me they are paragangliomas originating in a nervous system derived from the entoderm, a neurentoderm. Be that as it may, the chromaffinity of these cells permits us to suppose that, in addition to the fats, they secrete a substance more or less comparable to adrenalin but nevertheless different. We may suppose that this substance, poured into the

which take no part in the constitution of the carcinoid, namely, by those cells which I have thought to be *ganglionic*.

After penetrating the intramucous neuroma the carcinoid columns gain the filaments that traverse the muscularis mucosae, then Meissner's plexus, then Auerbach's plexus. They infiltrate without destroying the nerve fibers or the ganglion cells of the myenteric plexus and without provoking their increase. In the meantime, some of them get out of the nerve and invade the connective tissue and even the lymphatics.

This intranervous progress is not peculiar to carcinoids; it is seen also in epitheliomas of the digestive tract; but, in this event, the invasion of the nerve is secondary, it follows interstitial invasion. In carcinoids, on the contrary, the penetration of the nerve is primary while the connective tissue invasion is secondary. The name *neurocarcinoid* which I have proposed to give to carcinoids thus seems to me to be more justifiable than ever.

(b) Tracing the steps of this intranervous invasion we find that the corollary of the penetration of the various planes of the myenteric plexus is an overgrowth of the smooth muscle of the infested nervous region. To the invasion of the nerves of the muscularis mucosae there corresponds a myoma springing from it. If the carcinoid columns gain the nerves of the circular muscle and the submucous bundles depending on them, another myoma is added to the first. Similarly, when the carcinoid attains the external branches of Auerbach's plexus, the longitudinal muscle hypertrophies in its turn. If, in addition, the nerves of a muscle bundle of the meso-appendix are infiltrated, this bundle increases also. It should be noted that the overgrowth of the muscle is restricted to the area of nerve invasion; beyond this point it ceases quite abruptly.

Finally, we should note the chief point. When the carcinoid columns invade the nerves or the connective tissue of the visceral serosa or the fat of the meso-appendix, no muscle fibers are seen in their interstices. It follows that the muscle fibers observed in the "stroma" of carcinoids are not an integral part of these tumors. They result from proliferation of preëxisting muscle fibers, the nerves of which have been invaded by the carcinoid cells. The myogenic influence of the invasion of the nerves does not extend to a distance; it seems to exhaust itself on the spot. Moreover, it does not affect this or that muscle coat to the exclusion of the others, but it involves

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## DESCRIPTION OF PLATES

## PLATE 103

FIG. 1. Obs. 3 (123 m). Deep region of the mucosa.

On the left the muscularis mucosae "M." On the right, the tips of two crypts of Lieberkühn "LL." Between the muscularis mucosae and the tips of the crypts, lymphoid tissue and slender columns of carcinoid cells. These cells are not situated in the lymphoid tissue but in the filaments of the periglandular and subglandular plexuses, which they fill incompletely. The regions "N" where the nerves are not completely infiltrated show (1) that the collagenous sheath around the carcinoid column is none other than the sheath of the invaded nerve; and (2) that the nerve segments visible between two infested regions have undergone no degeneration. The continuity of the nerves, then, has not been broken by the invading cells.

FIG. 2. Obs. 3 (123 m). Middle region of the submucosa. The appendicular mucosa is on the right and above, the peritoneum on the left and below.

The anastomosing muscle bundles (dark gray) correspond to the cells of the muscular mechanism of the submucosa, locally hypertrophied. In the intervals, there is fibrous tissue (pale gray). The black spots represent carcinoid columns penetrating Meissner's plexus. None of them lies directly in the connective tissue. The muscle hyperplasia is limited strictly to the zone of infiltration of the nerves.

nerves, excites them and causes hypertrophy of the corresponding muscles.

This leads us back to a hypothesis formerly proposed by Ciaccio<sup>7</sup> of the rôle of the normal enterochromaffin cells. He believed that these cells secrete into the intestinal lumen a substance which, re-absorbed by the mucosa, played a part in the contraction of the intestinal muscle. What we have learned of the neurotropism of these cells enables us to modify this hypothesis somewhat. May we not suppose that their secretion, eliminated into the nerves of the mucosa (its limited diffusion is demonstrated by the localization of the carcinoid myomatoses), acts almost exclusively on the nerves of the mucosa and on the muscles of the muscularis mucosae?

#### SUMMARY AND CONCLUSIONS

1. Originating in the nerves of the mucosa, previously hypertrophied, carcinoids penetrate the myenteric plexuses progressively without destroying them and without provoking their hyperplasia. They merit the name of *neurocarcinoid*. Invasion of the connective tissue and of the lymphatics is secondary.

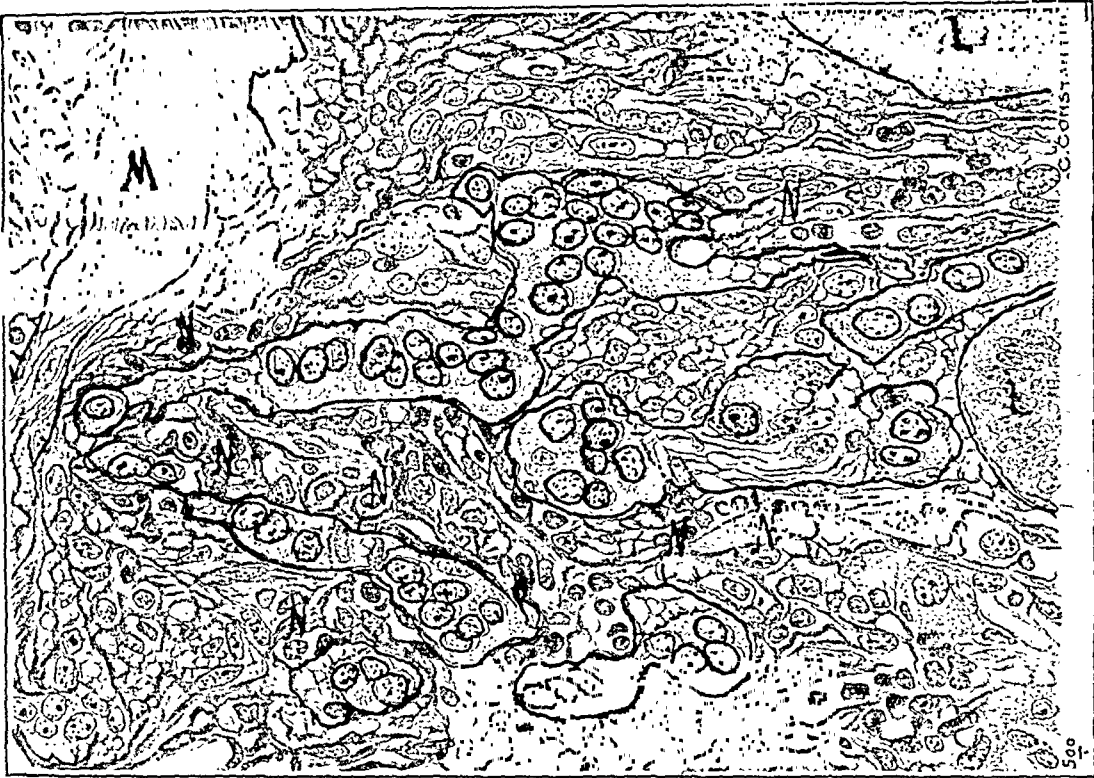
2. Connective tissue infiltrated by carcinoids does not originate muscle fibers. On the contrary, when the nerves are invaded by carcinoids, the corresponding muscle coats undergo hyperplasia restricted to the territory of the infected nerve. The muscle fibers formed in the interstices of carcinoids, then, are not an integral part of these tumors; they result from proliferation of preëxisting muscles provoked by the presence of argentaffin cells in their nerves.

3. This myogenic action of the argentaffin cells seems due to a product of limited diffusability secreted by the cells into the nerves (neurocrinia).

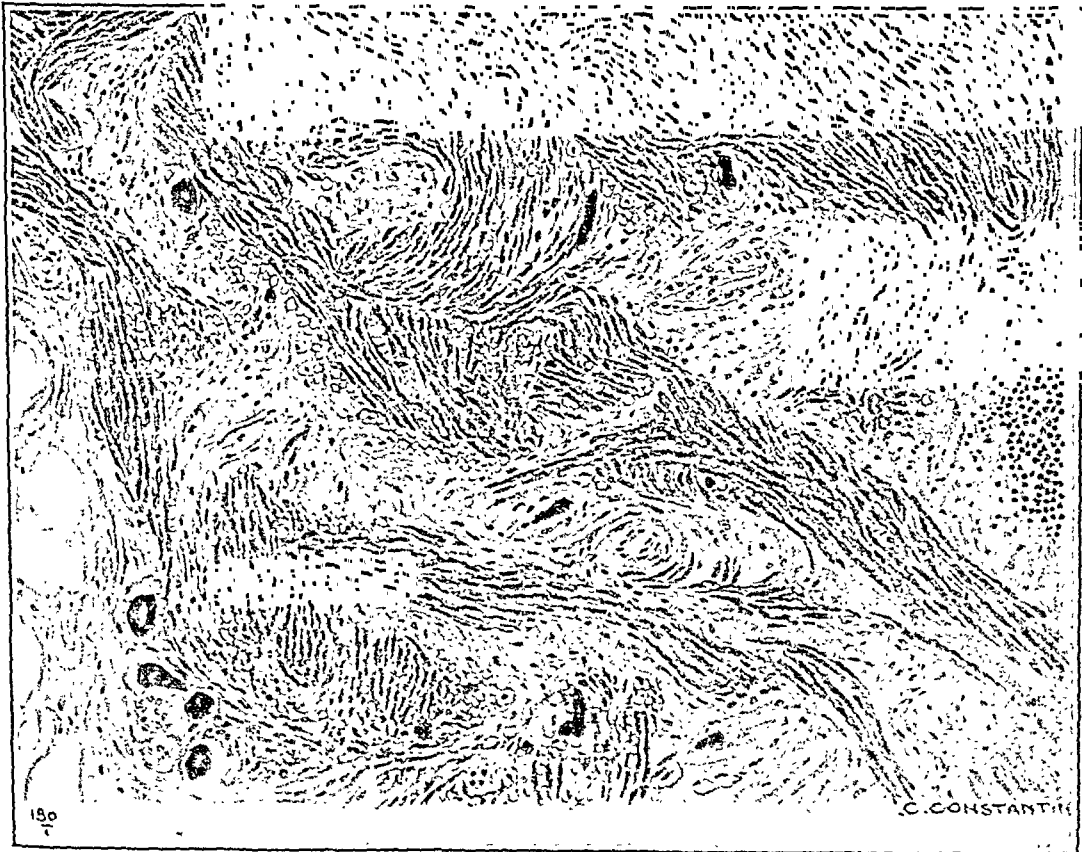
4. As a working hypothesis, one may suppose that the normal argentaffin cells of the intestinal mucosa function like the cells of carcinoids, and that their secretion poured into the plexus of the mucosa plays a rôle in the functioning of the muscularis mucosae.

I must again thank my friend, Dr. Laidlaw, for his translation of this paper for the Journal.





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PLATE 104

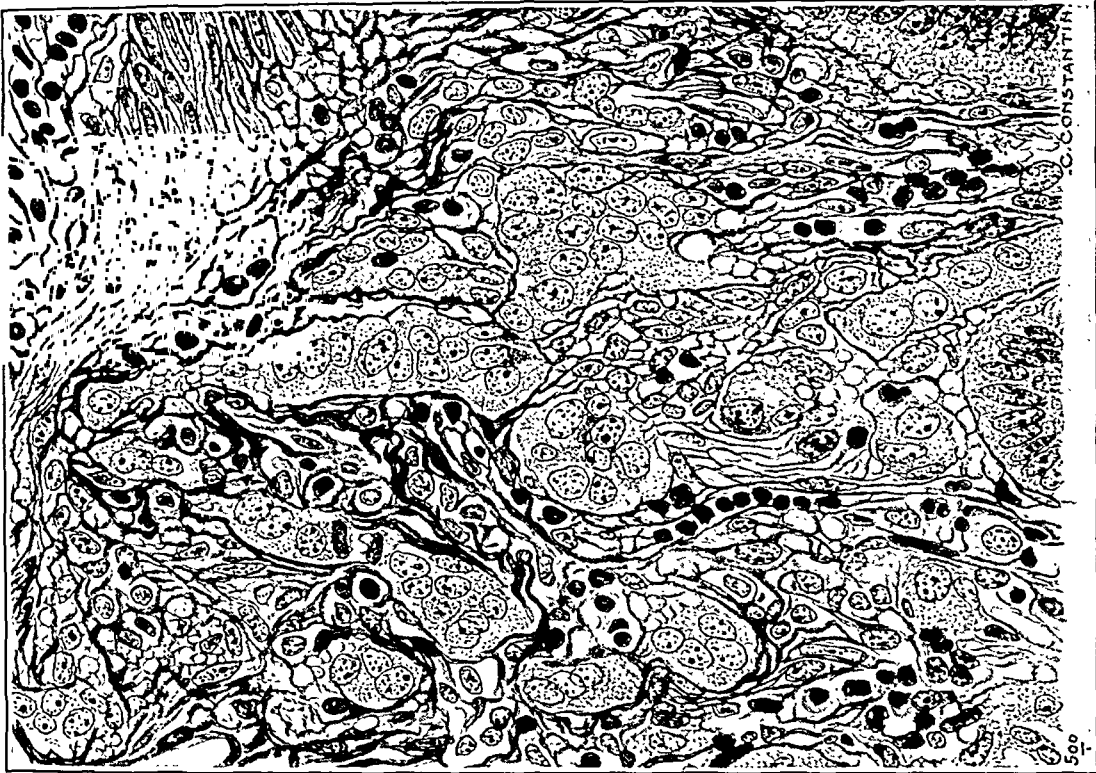
FIG. 3. Obs. 3 (123 m). Middle region of the myoma developed at the expense of the circular muscle.

The branches of the nerve plexus are injected by argentaffin cells, making them more prominent. The invasion does not involve the whole plexus but leaves free some of the branches "N," the slender ones. The cells, their contours indicated on the tracing by the dotted line, are ganglion cells of Auerbach's plexus. Despite the accumulation of argentaffin cells around them, these cells are perfectly intact. In the meshes of the plexus, there are many young muscle cells without definite orientation, probably arising from the circular fibers. On the right, lymphoid infiltration, the residue of recent inflammation.

FIG. 4. Obs. 3 (123 m). Region adjoining Fig. 3.

Node of Auerbach's plexus invaded by argentaffin cells. Three slender nerve branches "n" detach themselves. This node has no ganglion cells.

Observe the lack of orientation of the muscle fibers and the frequent presence of several nuclei in the same cytoplasmic spindle. The cells seem to multiply amitotically exclusively.



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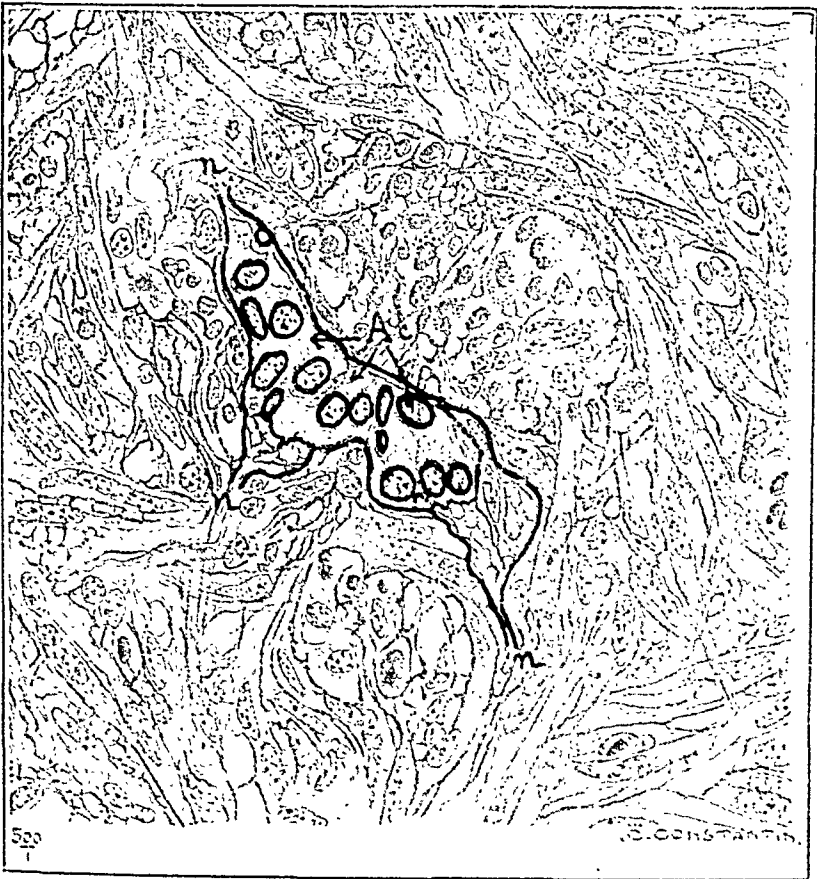


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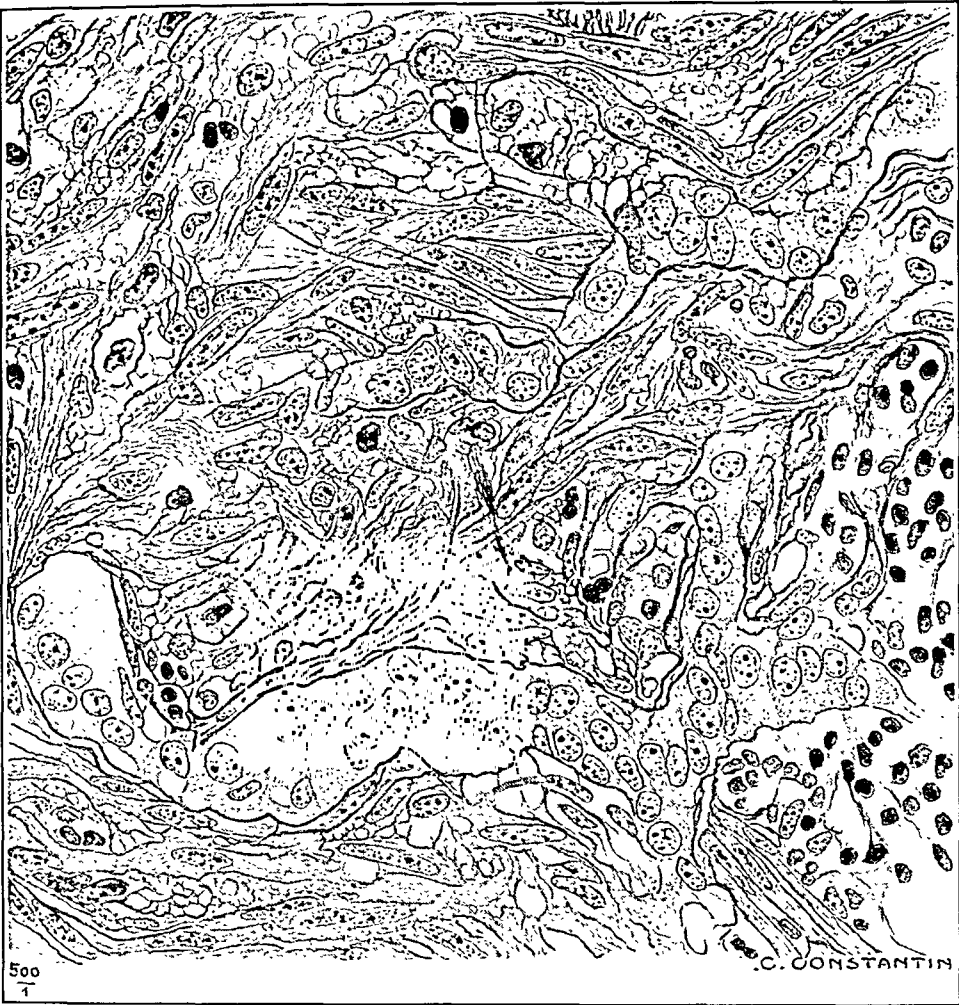


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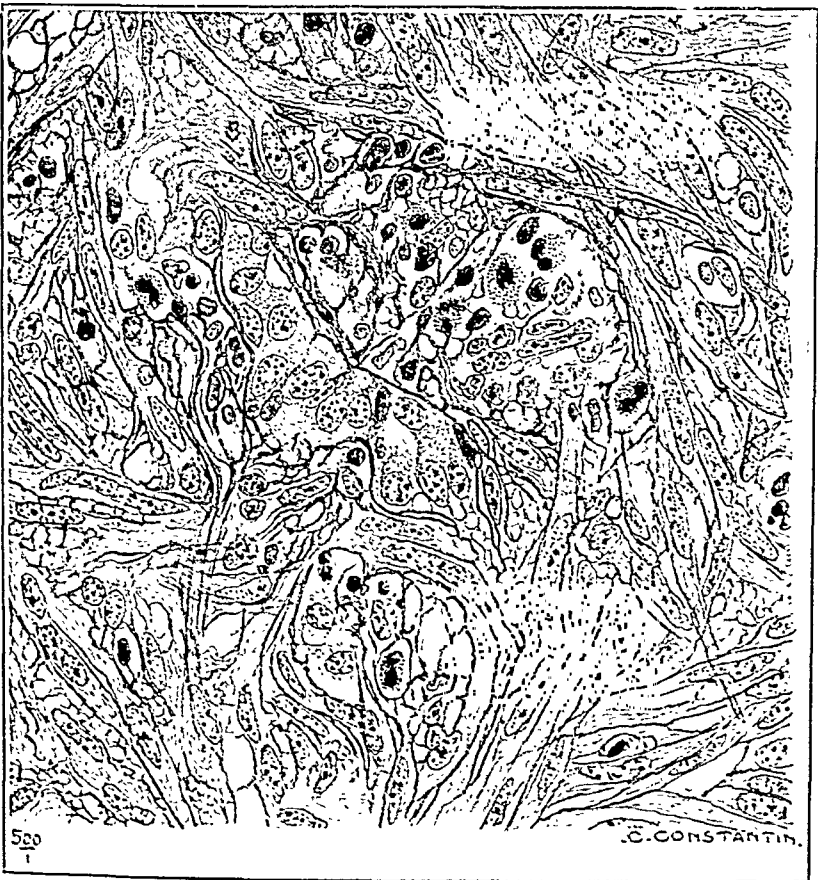


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gawa,<sup>10</sup> Danisch,<sup>11</sup> Forbus,<sup>12</sup> and Hamperl.<sup>13</sup> Hasegawa has shown, however, that the silver impregnation method has occasionally been irregular in tumors having the identical histological picture. One may say then that the silver impregnation method has shed much new light on these cells in their relationship to the cells in the crypts of Lieberkühn, although opinions still differ concerning the true nature of these tumors. The question of origin and malignancy remains unsettled, though several instances of metastasis have already been reported.

This report deals with a new case of carcinoid tumor of the small intestine with metastasis, observed at the Rochester General Hospital, which, it is hoped, may shed some light on the pathology and clinical significance of these tumors.

### REPORT OF CASE

*Clinical History:* A. F. F. (Hospital No. 50909), white, female, 74 years of age, admitted on April 15, 1929, had had frequent indigestion and anorexia over a long period. She had no trouble on the prescribed diet. In March, 1929, a constant pain developed in the back, and since that time the patient was confined to bed. She suffered from constipation. Physical examination was essentially negative. Some tenderness was noted, especially on the left side and in the epigastrium. The liver was palpable one finger's breadth below the costal margin. Roentgenograms showed a dilated small bowel. The patient died on April 23, 1929.

*Clinical Diagnosis:* Possible malignancy of gastro-intestinal tract, or head of pancreas.

### POSTMORTEM EXAMINATION

In the lower jejunum a tumor measuring 15 by 13 by 12 mm. was found situated in the wall directly opposite the mesenteric insertion (Fig. 1). The tumor bulged toward the lumen of the jejunum producing an almost complete obstruction. Only the tip of the enterotome was admitted through the lumen. The jejunum was much dilated above, and collapsed below, the tumor mass. In gross sections the mucosa and intestinal muscular layers were found to be infiltrated by the neoplasm. In this area the width of the intestinal wall measured from 5 to 10 mm. The serous layer was drawn into a "V" shape by the tumor and the opposite serous surfaces were adherent. In the mesentery, adjoining this region, a hemorrhagic tumor the size of a hen's egg was found. Hemorrhages were observed in the surrounding mesenteric fatty tissue, as well as in the peritoneum. The liver, corset-type in appearance, was slightly

## METASTASIZING "CARCINOID" TUMOR OF JEJUNUM \*

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Carcinoids are usually multiple tumors occurring in the small intestine, appendix, and occasionally in the colon. Histologically they are formed of epithelial cells arranged in communicating strands, irregularly sized and embedded in stroma. Lubarsch<sup>1</sup> in 1888 reported the first cases as primary carcinomas of the ileum. In 1907 Oberndorfer<sup>2</sup> applied the name "carcinoid" to these tumors. Although only about seventy cases comprise the total number of carcinoid of the small intestine, a considerable literature has dealt with their case reports, etiology, histopathology, physiology and ultimate prognosis. Before Masson's silver impregnation method of staining, most investigations followed purely morphological lines. By the silver method Lubarsch was able to demonstrate a definite relationship between the nests and columns of tumor cells with those in the crypts of Lieberkühn, thus suggesting the epithelial origin of these growths. At the same time he pointed out the distinct difference between these cells and those of adenocarcinomatous tumors occurring in other parts of the gastro-intestinal tract. The opinion of Lubarsch was shared by Bunting,<sup>3</sup> Burckhardt,<sup>4</sup> Versé,<sup>5</sup> and Krompecher,<sup>6</sup> while Saltykow,<sup>7</sup> Oberndorfer,<sup>2</sup> and Toenniessen<sup>8</sup> failed to demonstrate any evidence in support of the intestinal epithelial origin of these cells. Because of their structural similarity to the islands of Langerhans and their multiple occurrence, Saltykow held them to be derived from misplaced embryonic islands of Langerhans. Oberndorfer, on the other hand, considered them embryonal malformations, while Toenniessen asserted that they were misplaced, heterotopic and spread-out intestinal epithelium. By means of the silver impregnation method Masson<sup>9</sup> found that besides being chromaffin in nature, as was pointed out by Oberndorfer, the cells of the carcinoid tumors reduced solutions of ammoniacal silver in the same manner as the so-called Nicholas-Kulchitzky-Schmidt cells of the intestinal epithelium. Hence he called the carcinoids "argentaffin cell tumors." His contention has been confirmed by Hase-

\* Received for publication May 9, 1930.



kühn (Figs. 2 and 3). Again, these tumor cells showed a tendency to form branches interconnecting the many tumor masses and all traceable to the same gland of Lieberkühn. Fig. 4 demonstrates how sometimes these tumor cells would fill the intravillous spaces, thus coming in direct contact with the intestinal lumen. Frequently the tumor cells would penetrate into the submucosa, forming in this layer much larger masses, covered toward the periphery by unaltered mucosa in which no tumor cells were found. Two hundred serial sections, each about 5 microns in thickness, prepared from the edge of the tumor, failed consistently to show any tumor cells in the mucosa. The circular and longitudinal layers of muscular fibers, however, as well as the serosa, were well infiltrated by the tumor cells, which assumed the shape of thin bundles in the muscular layers, and nodular forms as large as 4 to 5 mm. in diameter, in the serosa.

The stroma of the tumor was made up of loose connective tissue, hyalinized in many areas. Smooth muscle cells could be found in the vicinity of blood vessels. Moderate infiltration of round cells was present in the upper half and in the ulcerated parts of overlying mucosa. Few blood vessels occurred within the tumor, but toward the periphery they became more numerous and more dilated. Tumor cells were found within the blood vessel lumina (Fig. 5).

Sections made from the metastatic nodule in the mesentery showed a large central necrotic area. This was surrounded by fibrosis and blood vessels, while toward the periphery a wide layer of tumor cell masses was embedded in a stroma of hyaline connective tissue. In this area numerous blood-filled sinuses occurred (Fig. 6). The sinus walls had no endothelium and seemed formed entirely of tumor cells. In this area also, the blood vessels were invaded by masses of tumor cells.

The lymph node in the neighborhood of the mesenteric tumor nodule showed masses of metastatic tumor cells. Especially toward the periphery, the dilated blood and lymph vessels were filled with tumor cells (Fig. 7). These vessels retained their endothelial lining.

Sections of a cherry-sized metastatic tumor nodule in the liver showed central necrosis, while at the periphery were found numerous large and freely anastomosing masses of tumor cells (Figs. 8 and 9). The blood vessels, which occurred mostly at the periphery, contained masses of tumor cells.

atrophied. On its surface a few grayish white, firm foci were noted which measured 6 to 8 mm. in diameter. These foci corresponded in situation to the metastatic tumors immediately below the liver capsule. On section a few smaller nodules of metastatic tumors were found deeply embedded in the liver. About 15 cm. below the above described jejunal tumor was a smaller tumor, measuring 3 by 4 by 4 mm., and 10 cm. below this mass another tumor 2.5 by 2 by 2 mm. in size was found in the submucosa opposite the insertion of the mesentery.

The autopsy revealed further a generalized arteriosclerosis with arteriosclerotic kidneys, brown atrophy of the heart, and slight pulmonary edema. The cause of death was given as carcinoma of the small intestine, with intestinal obstruction.

#### HISTOLOGICAL EXAMINATION

*Large Tumor Nodule:* Numerous serial sections, 5 or 6 microns thick, were prepared from several blocks. A considerably thickened mucosa surrounded the tumor. Centrally, the tumor contained an ulcerated area 2 by 3 mm. in diameter, which extended to the muscularis mucosae layer. The peripheral portion of mucosa overlying the tumor appeared normal or slightly compressed. The papillae of the mucosa were distinctly seen in the peripheral portion as well as in the central parts, although centrally they seemed compressed, flattened, and not clear-cut, indicating superficial necrobiosis. Approximately one-third of the surface area of the mucosa covering the tumor showed definite thickening, which corresponded to the middle third of the tumor mass. Sections from this area revealed glands of Lieberkühn in the superficial layers of the mucosa. The mucosa below these glands was occupied by a great number of tumor cell nests. In general, the glands failed to show signs of compression, although a few such compressed glands did occur. Wherever the columns of tumor cells were scarce, the glands of Lieberkühn occurred with regularity in the deeper parts of the mucosa. Certain changes were noted in the glands of Lieberkühn not usually observed in normal glands, and the cells, instead of occurring in one row, were irregularly arranged. Among these were found many smaller cells possessing each a darkly stained nucleus. These cells preponderated in the basal portions of the glands and seemed characteristically identical with the tumor cells occurring outside the glands of Lieber-

curred in the submucosa where it occupied a much wider area than in the mucosa. The muscular fibers likewise showed infiltration by masses of tumor cells, presenting the same features as already described in the first tumor, although hyalinization of the stroma was not marked.

The small tumor of the jejunum, which measured 2.5 by 2 by 2 mm., presented a much thickened mucosa, corresponding to that of the tip. Immediately adjoining the tumor, the mucosa was somewhat thinned out, but further on it resumed its normal appearance. In the thick portion many columns and masses of tumor cells appeared. The course of the tumor columns seemed perpendicular and running in the same direction as the crypts of Lieberkühn, which were cut lengthwise. Cross-sections of the glands of Lieberkühn, which appeared in several places in this area, did not indicate any pressure. These glands occasionally occurred in direct contact with the tumor cells which had broken through the muscularis mucosae layer (Fig. 12). Fortunately, a section was made directly into a gland of Lieberkühn which emptied into the intestinal lumen; in its lower half it showed an immediate contact of the tumor cells with the apparently normal cells of the crypt (Figs. 10 and 11). In the few sections which followed, only a cross-cut of the gland was obtained, but in these sections the original column of tumor cells was found to have broken through the muscularis mucosae layer into the submucosa.

In several similarly prepared pictures, a direct contact was found to exist between the tumor cells and the glands of Lieberkühn. The bulk of the tumor rested in the submucosa, although a few cells had reached the muscularis mucosae layer. No round cell infiltration was observed in the tumor stroma in the mucosa. Numerous capillary blood vessels were found in the submucosa. No apparent difference could be detected in these cells from those occurring elsewhere. The silver impregnation method of staining was not carried out in the two smaller tumors because of their previous fixation in formalin.

#### DISCUSSION

The jejunal tumors described obviously correspond with the small tumors first reported by Lubarsch and subsequently called "carcinoids" by Oberndorfer. Their occurrence in an elderly individual, their multiple nature, the sharp histological difference

The morphology of the encountered tumor cells agreed in the main with that described by numerous investigators, and in particular with Masson's excellently described studies. Thus the cells appeared round or polygonal, palisade, columnar or prismatic, all varieties of the same species according to Masson. Close to their origin in the glands of Lieberkühn, the round cell type dominated. The palisade cells, inconstantly present, occurred in the irregular columns of tumor masses. The prismatic cells were found around small cavities which sometimes contained small amounts of homogeneous material. The nuclei of the tumor cells varied between round, oval or spindle-shaped forms, all staining very deeply. Mitotic figures were not observed, although carefully sought for. Sections stained by the hematoxylin-eosin method showed the cytoplasm of the tumor cells to be filled by minute acidophilic granules — chromaffin and argentaffin granules. The sections were stained by Masson's and the blocks by Hasegawa's silver impregnation methods. Positive results were obtained by both methods. The sections stained by Masson's method showed the silver-reduced granules as dark brown in color and exceedingly fine. This method preserved the entire structure very well and produced excellent demonstrating material.

The tumor cells in the metastatic nodules were identical with those found in the primary tumor and yielded positive results by the silver impregnation method. The tumor cells in the metastatic areas in the liver seemed slightly larger, the nuclei more uniformly round or vesicular, a condition perhaps explainable by a somewhat reduced pressure. Complete absence of mitotic figures was noted in these cells.

Four hundred serial sections were prepared from the second and third tumor nodules found in the jejunum. Because of superficial necrobiosis, the second tumor was found unsuitable for etiological study. The overlying mucosa seemed thicker at the tip of the nodule, where columns of round or elongated tumor cells occurred. Longitudinal or cross strands of crypts of Lieberkühn could be seen. Occasionally a column of tumor cells was located between the villi, thus coming into direct contact with the intestinal lumen. The overlying mucosa near the tip was thinned out, but further on regained its normal appearance. The muscularis mucosae was invaded by tumor cells in many places. The bulk of the tumor oc-

ever, because of the already mentioned disappearance of the lining cells.

Mention has been made in literature that the mucosa seemed to be intact especially above smaller carcinoids. From the reports we are unable to ascertain whether or not serial sections were made of these small tumors. In our case, we were fortunate to demonstrate the retention of intestinal mucosa in the apices of the tumor masses. Sections made towards the periphery, however, revealed the presence of tumor cells only in the submucosal and muscularis layers.

By means of the silver impregnation methods suggested by Masson and Hasegawa, we feel that our findings further substantiate the hypothesis concerning the glands of Lieberkühn origin of these argentaffin tumor cells; the so-called Nicholas-Kulchitzky-Masson cells normally contain the argentaffin, silver-reducing granules. It seems, therefore, quite proper to call the carcinoids by the name "argentaffin cell tumors." We believe, however, that the appellation "carcinoid" is more suitable in consideration of the tendency of these tumor cells to infiltrate and eventually to become malignant. Even the smallest nests of these cells possessed ability to infiltrate the muscular layers. On this basis our opinion is in complete agreement with those who hold that carcinoids should be classified as true carcinomas. Their proliferation is slow, but the formation of metastases is only a question of time. Metastases take place mainly through the blood stream, although occasionally dissemination may occur by means of the lymph vessels. We have already demonstrated the abundant presence of tumor cells along the walls of the dilated blood vessels and the gross hemorrhages which gave the characteristic hemorrhagic appearance to the large metastatic tumor found in the mesentery. Versé also reported metastases both through blood and lymph vessels, for in two cases he noted tumor cells in the mesenteric veins, and in both the blood and lymph vessels of a mesenteric lymph gland. Burckhardt also found small groups of tumor cells among the blood cells in a mesenteric vein.

Metastasizing carcinoids of the small intestine have been described by Ransom,<sup>14</sup> Versé,<sup>5</sup> and Schopper.<sup>15</sup> The case reported in this paper should be added to these already recognized metastatic carcinoids.

Very little can be said about the actual etiology. Several investigators found chronic inflammatory processes in the intestinal tract associated with carcinoids. It is possible that a direct relationship

from adenocarcinomas, and their histological identity with known carcinoid tumors of the small intestine, confirm their identity as such. Of greatest interest to the histopathologist is the origin of these tumor cells, their infiltrating character, and their tendency to form carcinoid metastases. Because of the disagreement among investigators about the origin of carcinoid tumors, a special attempt was made to prepare a large series of sections in order better to trace their origin and their course of metastases. It is evident from Figs. 2 and 3 that the tumor cells accumulate at the tip of the glands of Lieberkühn within the basal membrane. The tumor cells are found immediately beneath the lining cells and in intimate contact with the Lieberkühn glands. The serial sections reveal that the basal membrane is being pushed out by the tumor cells in a manner similar to the budding described by Masson in a case of appendical carcinoids. During the early stages of development, these budding forms do not appear to break through the basal membrane, and in some sections they fail to unite with columns of tumor cells. Other buds, however, have perforated the basal membrane and can be traced into the deeper layers.

It is also observed that several columns of tumor cells originate in a Lieberkühn gland and by the formation of ramifying columns the tumor steadily increases in size. It is probable that only a definite number of glands of Lieberkühn give rise to a tumor. This fact is suggested by the constant observation that columns of tumor cells occur only in the apex of the tumor nodule. The rough pyramidal shape of the tumor nodules may be explained by the abundant ramification and proliferation of the cells into the deeper layers. In a number of glands the lumina are filled with tumor cells. It is evident that the course of tumor proliferation within the glands is toward the intestinal lumen, and hence the lining cells of the glands disappear. If only slight pressure is exerted from the side by the growing tumor mass, a direct opening into the intestinal lumen may be found. If, on the other hand, pressure is brought to bear from below and from the side, the villi become compressed and their openings disappear. For this very reason it is essential to make serial sections in order to find convincing evidence in support of the origin of these tumor cells from the glands of Lieberkühn. It is probable that some of the perpendicular columns of tumor cells found in the intestinal mucosa occupied the place of the glands of Lieberkühn. No clear-cut proof of this contention is possible, how-

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## DESCRIPTION OF PLATES

### PLATE 105

- FIG. 1. Carcinoid tumor 15 by 13 by 12 mm. in size, which caused intestinal obstruction and metastases in the mesentery and liver.
- FIG. 2. Lieberkühn gland in cross-section from the large jejunal tumor. A few tumor cells lie beneath the lining cells of the gland. They form a definite group within the pushed-out limiting membrane.
- FIG. 3. The same gland as in Fig. 2 as it appears in the following serial section. The tumor cell group is somewhat larger.
- FIG. 4. Tumor cells filling the space between villi of a Lieberkühn gland.
- FIG. 5. Large tumor cell group in a blood vessel of the carcinoid tumor of the jejunum, 15 by 13 by 12 mm. in size.
- FIG. 6. A section from the metastatic nodule in the mesentery. Numerous wide sinuses filled with blood and surrounded by tumor cells.

exists between chronic irritation and carcinoids, although we failed to demonstrate any round cell infiltration in the mucosa adjoining the smallest tumors in our case. The invasion of round cells into the mucosal part of the large tumor seemed secondarily to be due to ulceration.

Saltykow believes that carcinoid tumors are of no clinical importance and are only accidentally discovered at autopsy. His conclusion seems unwarranted, inasmuch as the seventh case reported by him presented a carcinoid tumor in the ileum, measuring 2.5 by 2.5 by 1.5 cm., occurring 6 cm. above the ileocecal valve and causing almost complete obstruction, with corresponding clinical symptoms. Judging from the history of his case, this tumor was the actual cause of the patient's death. In our case, the tumor, which measured 15 by 13 by 12 mm., had probably caused intestinal disturbance for some time, until it finally brought about almost complete obstruction. Roentgenograms had shown dilated loops of the small intestine, and at autopsy the carcinoid tumor was found in the position indicated by the films. It is certain that had the patient sought hospital care earlier, resection of the involved part of the jejunum might have saved the patient's life and prevented metastases. It appears, therefore, that carcinoid tumors may occasionally assume clinical significance.

#### SUMMARY

1. A case of multiple carcinoid tumors of the jejunum, one of which caused intestinal obstruction, with metastases in the mesentery and liver, is reported.
2. Histological examination revealed the picture of typical carcinoid tumors. Serial sections definitely indicate that at least two of the tumors originated in the crypts of Lieberkühn.
3. Positive silver impregnation of the tumor cells confirms Masson's contention that the origin of carcinoid tumors is in the Nicholas-Kulchitzky-Masson cells in the glands of Lieberkühn.
4. Carcinoid tumors may assume clinical significance.

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PLATE 106

- FIG. 7. Metastatic tumor cell groups in the lymph sinuses of a mesenteric lymph gland.
- FIG. 8. Section from a metastatic nodule in the liver.
- FIG. 9. The extreme periphery of a metastatic liver tumor nodule.
- FIG. 10. Section from the smallest carcinoid tumor of the jejunum. On the right side a Lieberkühn gland is cut longitudinally and empties into the lumen of the intestine. A tumor bundle originates in the gland and fills out a part of the gland lumen.



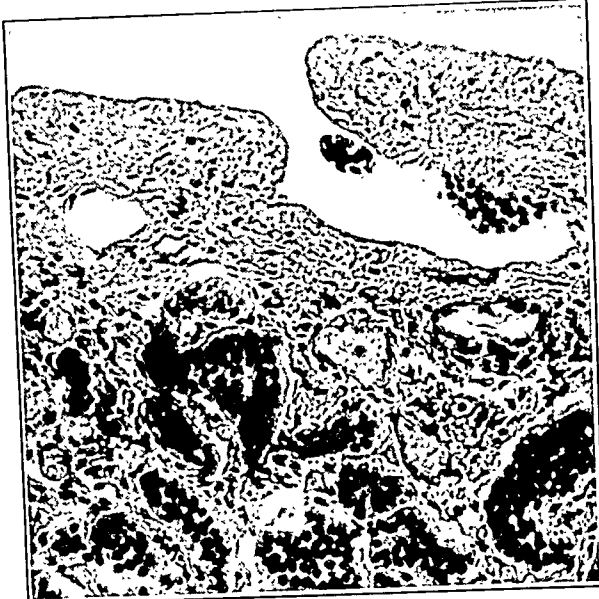
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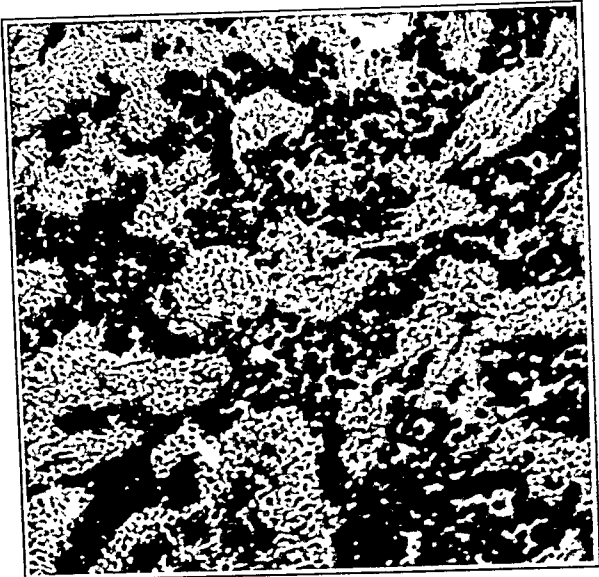
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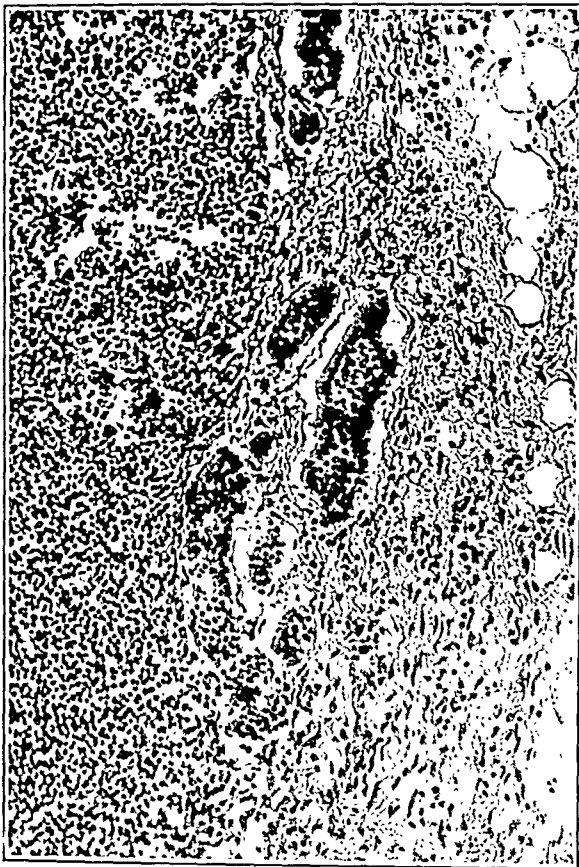
Gáspár

Metastasizing "Carcinoid" Tumor of Jejunum

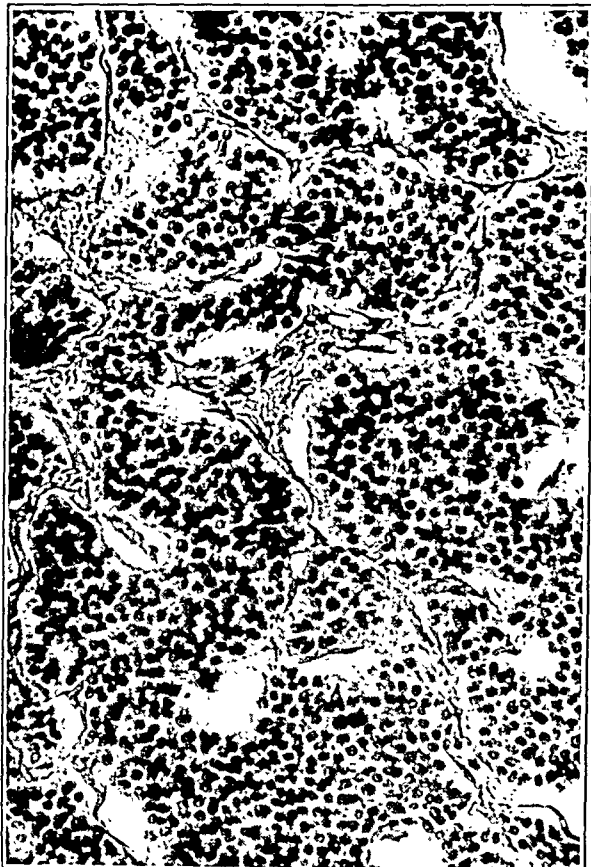
PLATE 107

FIG. 11. Higher magnification of Fig. 9. The origin of the tumor cells from the Lieberkühn gland is quite convincing. The tumor cells are in immediate contact with the cells in the crypt and fill its lower portion.

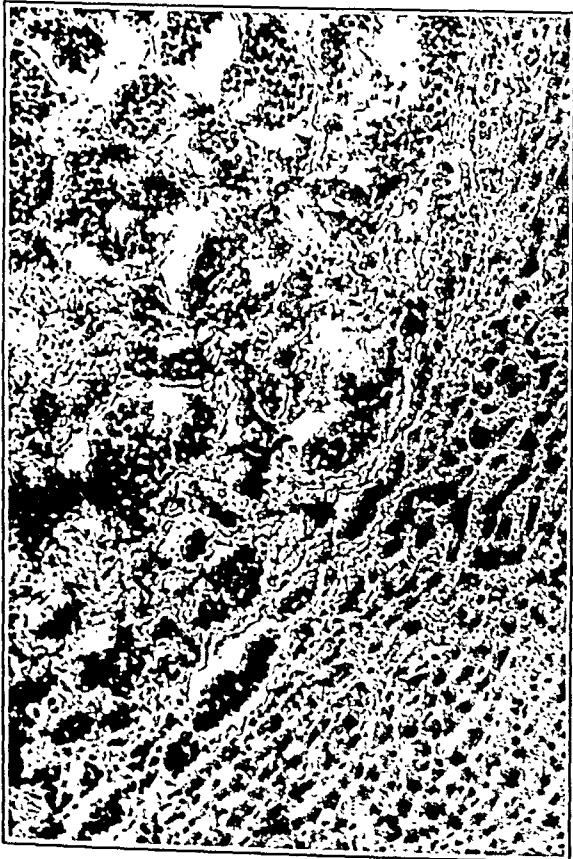
FIG. 12. Section from the smallest jejunal tumor. Above the mucosae muscularis two Lieberkühn glands are cut across. On the left side, the Lieberkühn gland gives rise to a column of tumor cells which breaks through the mucosae muscularis. Branching of the same column can be made out.



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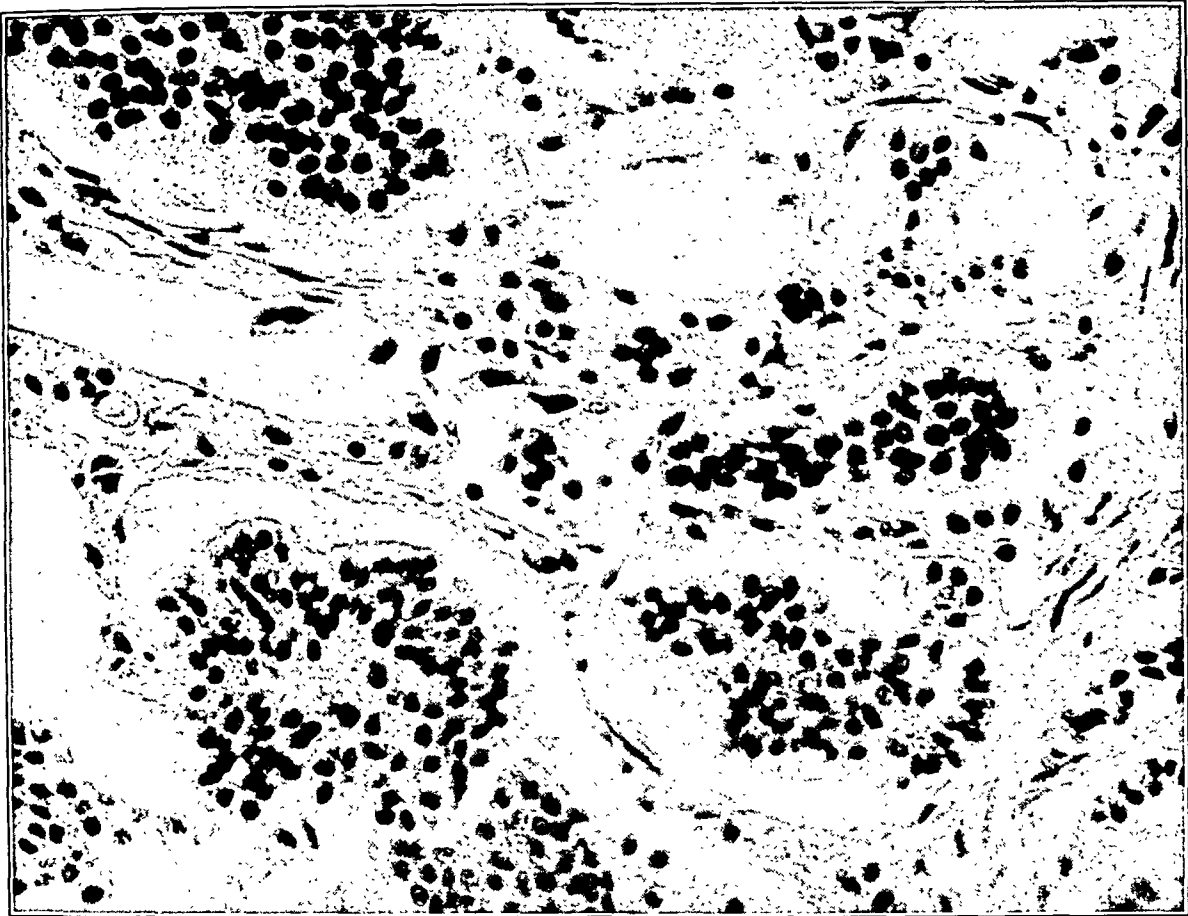


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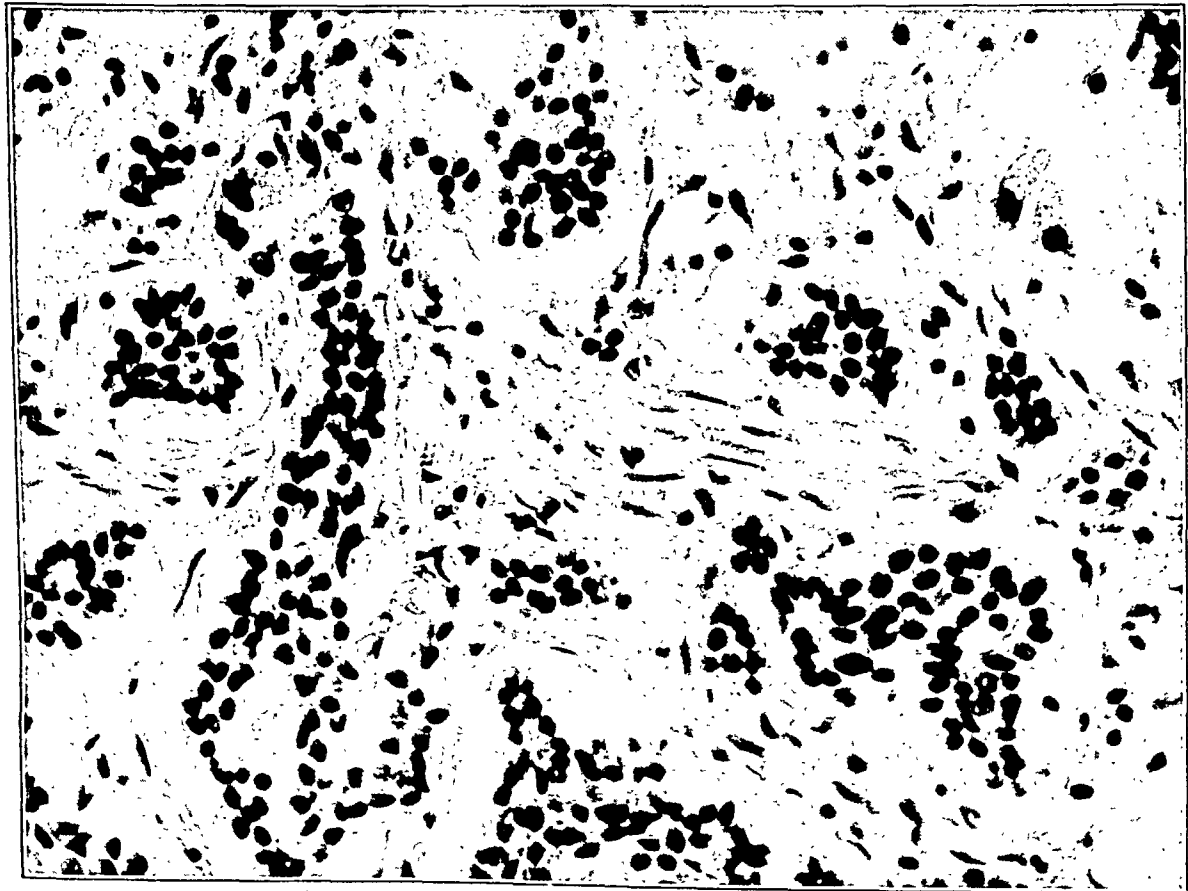
Gáspár

Metastasizing "Carcinoid" Tumors of Jejunum





II



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that expressed by Mallory and Parker who deny formation of reticular fibrils by ordinary capillary endothelium, and assert that it is produced only by fibroblasts. The black impregnated fibrils are said to be fibrils originating in fibroblasts taking a black impregnation reaction due to a fine dispersion of the fibrillar substance.

Most of the other contributions deal with the occurrence of reticulum in various organs and tissues under normal and pathological conditions, without positive statement of its derivation.

Without further reference at this time to the literature, the method we have employed, our findings and their interpretation will be set forth.

### THE METHODS EMPLOYED

Kinney <sup>4</sup> (1928) briefly reported the observation, that, in tissues fixed in a solution of 4 per cent formaldehyde containing 1 per cent sodium sulphantimonate, a substance was impregnated which she considered to be reticulum. This fixation was applied to tissues by the writer and a large variety of counterstaining methods applied. Results gave sufficient encouragement to pursue the subject further. Finally a silver ammonium carbonate solution was employed in tissues so fixed and a surprisingly sharp impregnation of reticulum was observed. Various refinements were devised. The following methods of fixation, impregnation and counterstaining were adopted which yielded the most satisfactory sections. These methods were employed in this study.

*Fixation:* Primary fixation in 4 per cent formaldehyde, preferably neutral, or Kaiserling's solution No. 1 is used. Following this, tissues are treated essentially as in the Kaiserling method of preservation for museum specimens. The tissues are washed in running water 24 hours, passed into 80 per cent alcohol for 24 hours, and then into Kaiserling's solution No. 3 for 3 or more days. Satisfactory preparations may be obtained in sections that have been long preserved in formalin or in Kaiserling's solution No. 3. Sections are taken from the Kaiserling solution and washed in running water for 12 to 24 hours. After washing, sections cut properly for paraffin blocks, 2 to 3 mm. in diameter, are mordanted or refixed in a freshly prepared 0.5 per cent solution of sodium sulphantimonate made in a 4 per cent solution of neutral formaldehyde. This refixation is employed for 24 to 48 hours. After this mordanting, sections are again washed

RETICULUM. ITS ORIGIN. THE OCCURRENCE OF RETICULUM  
FIBRILS IN CAPILLARY ENDOTHELIUM. A NEW METHOD OF  
DEMONSTRATION. II. THE FINER CAPILLARY BED \*

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The present report is the first of a series of studies dealing with the derivation and differentiation of supportive substances from the mesenchyme as revealed by a new method of metallic impregnation described in detail below. Particular attention is devoted to the origin of reticulum and its occurrence in the mature organism. Other phases of mesenchymal differentiation are reserved for further study. In a preliminary way, fully realizing factors of incompleteness, a new concept of the finer capillary bed is presented.

A considerable literature has accumulated upon the subject of reticulum since Mall<sup>1</sup> in 1891 announced his discovery that the framework of many organs and tissues of the mammalian body is composed neither of white fibrous connective tissue nor of yellow elastic tissue, but of a third type of supporting substance composed of fine interlacing fibrils which not only differ from the white fibrous tissue in appearance, but are more resistant to acid and alkaline solvents, and are not so readily attacked by digestive ferments.

The introduction of the Bielschowsky method of silver impregnation stimulated renewed interest in reticulum. No attempt will be made in the present paper to review the rather extensive literature upon this subject. Some idea of its unsettled status may be gained by citing the contrasting concepts of Corner<sup>2</sup> (1920), and Mallory and Parker<sup>3</sup> (1927). Corner described reticulum fibrils arising in and extending from the capillary endothelial cells of the corpus luteum, adrenal, hypophysis and kidney. This author is one of the few making the distinct assertion that capillary endothelial cells in the adult organism produce a fibrillar substance, and identifying this substance with reticulum because of its affinity for impregnation with silver by the Bielschowsky method. Opposed to this view is

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*Staining Results:* Mesenchymal fibrils range from yellow and golden brown to black. Reticulum fibers black. Collagen fibers golden brown (transition colors seen).

*Method II. With Gold Toning*

1. Remove paraffin from sections and place in distilled water in usual manner.
2. Impregnate with silver ammonium carbonate (freshly prepared each time and discarded) for 30 minutes in oven at 37° C.
3. Wash sections in distilled water about 8 to 10 consecutive changes.
4. Immediately pour on 4 per cent *neutral* formaldehyde, let stand for 5 minutes to reduce silver.
5. Rinse well in distilled water.
6. Tone in aqueous gold chloride solution 1:250 (acid yellow gold chloride) for 5 minutes.
7. Rinse well in distilled water.
8. Pour 5 per cent oxalic acid on sections for 5 to 8 minutes.
9. Wash well in distilled water.
10. Fix with 5 per cent aqueous solution sodium hyposulphite. Change the hyposulphite as many times as necessary until solution is clear.
11. Rinse in distilled water and wash at tap 4 to 6 hours before counterstaining.

*Staining Results:* Mesenchymal fibrils, delicate rose red to black. Reticulum fibers, black. Collagen fibers, rose red.

*Counterstaining:* Counterstaining by the May-Grünwald-Giemsa method has given the most satisfactory results. The method is essentially similar to that employed by Downey<sup>7</sup> in his studies of developing lymph nodes.

1. Place sections 15 minutes in dilute acetic acid (6 drops per 100 cc.).
2. Place sections 15 minutes in distilled water.
3. Stain in equal parts of saturated methyl alcohol solution of May-Grünwald eosinate of methylene blue, and distilled water 1 to 2 minutes.
4. Rinse sections in distilled water.

in running tap water for 24 hours, then passed into 80 per cent alcohol, dehydrated in the usual manner by running through 95 per cent and absolute alcohol, cleared in xylol and embedded in paraffin. Sections are cut the desired thickness and impregnated in the following manner.

*Impregnation:* Two methods of impregnation may be used, one involving gold toning, the other not. Both methods are detailed below. Either method gives satisfactory results. Toning transforms the golden brown reaction of collagen in silver sections into a rose red.

The silver impregnation method is essentially that devised by Foot for paraffin sections and detailed in McClung's "Microscopic Technique."<sup>5</sup> All preliminary treatment with sodium hyposulphite, potassium permanganate and oxalic acid, are, however, omitted. The gold toning employs a modification advised by Laidlaw<sup>6</sup> in the use of oxalic acid after the gold chloride toning bath.

#### *Method I. Without Gold Toning*

1. Remove paraffin and place sections in water in usual manner.
2. Impregnate with silver ammonium carbonate solution (prepared as described in footnote \*) for 30 minutes in oven at 37° C.
3. Wash sections in distilled water — about 8 to 10 consecutive changes.
4. Immediately pour on 4 per cent *neutral* formaldehyde and let stand for 5 minutes.
5. Rinse sections in distilled water several times.
6. Fix in 5 per cent sodium hyposulphite 2 to 5 minutes.
7. Wash at tap for 4 or more hours.

#### *\* To Prepare Silver Ammonium Carbonate.*

1. Mix 12 cc. each of 10 per cent solution of silver nitrate and a saturated solution of lithium carbonate.
2. Wash precipitate 5 or 6 times with about 75 cc. of distilled water.
3. Add about 50 cc. of distilled water to washed precipitate and dissolve precipitate by adding ammonium hydroxide drop by drop until solution is almost clear. (Do not add too much — leave a few granules undissolved.) About 12 to 15 drops of ammonium hydroxide are needed.
4. Add distilled water to make solution of 85 to 100 cc. and filter.

*Note:* Solution must be prepared fresh for each batch and should be discarded after use. The solution is unstable and if exposed to the combined action of light and alcohol, there is danger of formation of an explosive mixture.

been traced with comparative ease using the method described. This will be the subject of a subsequent report.

*Differentiation of Fibrous Tissue in the Mesenchyme:* The morphological details of the differentiation of mesenchyme into fibrous tissue are so intimately associated with the problem in hand that an outline of this phase is deemed essential. As previously indicated, the delicate fiber substance of the simple mesenchyme shows shades ranging from yellow to black in untuned sections, and delicate rose to black in toned sections. With a condensation of fibrillar substance the color becomes more distinctly golden brown in simple silver prepared sections, and rich rose red if followed by gold toning (the color reactions of collagen). The transformation of simple mesenchyme into fibrous tissue entails a realignment of cells and fibrillar substance with the alteration in impregnation reaction indicated. The differentiation undoubtedly follows definite mechanical principles. For example, a bronchus enlarging in the mesenchyme acquires at its periphery a collagenous reacting mesenchyme—that is fibrous tissue. The fibers assume a radial arrangement about the expanding structures, the nuclei elongate in the direction of the fibers, the fibers now become more closely grouped, and the impregnation reaction becomes the distinct golden brown of collagen. The same phenomenon has been observed in the developing cuspid valves, in the corium and in other places where permanent fibrous tissue is being laid down. Fig. 1, a section of the skin and subcutaneous tissue of a 7.5 cm. pig embryo shows the transformation of the mesenchyme into fibrous tissue in the position of the corium. The loose underlying tissue is the fibrillated undifferentiated mesenchyme. Fig. 2 shows the same in greater detail.

*The Development of Capillaries and Distal Lymphatics in the Mesenchyme:* While not the primary consideration of this report, observations on the formation of distal lymphatics appear so clear-cut that brief attention will be given them at this time. Studied in the interlobular septae of the lung, these appear as simple clefts in the mesenchyme shown under low power magnification in Fig. 3. In Fig. 4 a portion of the wall of a lymphatic is shown in greater detail. The lining cells show fibrillated processes anastomosing freely with those of the surrounding mesenchyme. The nuclei flatten, paralleling the lymphatic wall and the fibers coarsen slightly by condensation.

5. Giemsa stain diluted 1:15 for 15 minutes.
6. Wash in distilled water.
7. Differentiate in dilute acetic, 6 drops to 100 cc. for  $\frac{1}{2}$  to 2 minutes. (This differentiation should be watched under the microscope.)
8. Rinse in distilled water.
9. Dehydrate rapidly in acetone.
10. Clear in cedar oil, 1 minute.
11. Xylol.
12. Mount in thickened cedar oil.

A simpler, yet satisfactory counterstain, is the routine hematoxylin and eosin.

The above described methods have in the writer's hands given clean sections with clear differentiation. Connective tissue fiber substances including reticulum are completely impregnated and a satisfactory polychrome counterstain secured. In addition to applying the method to adult human tissues, both normal and pathological, the method has been employed in a small series of pig embryos of 7.5, 11 and 22 cm. This has greatly helped in achieving an understanding of reticulum in the normal fully developed human organism.

### THE MESENCHYME

The mesenchyme consists of cells with round, oval or slightly irregular nuclei usually surrounded by a small amount of faintly staining cytoplasm (the endoplasm) from which radiate delicate fibrils and fibers which are impregnated by the method employed. An exceptionally rich fibrillar mesh is formed, the fibrils of adjacent cells readily anastomosing with one another. These fibrils impregnated with silver alone range from a yellowish or golden brown color to black, and in toned sections from a delicate rose red to black. At nodal points where the fibers cross, the depth of the tone is increased. Mall<sup>8</sup> (1902) showed, that in the intestine, reticular fibrils develop in the cytoplasm of the mesenchymal syncytium and later Hueck<sup>9</sup> (1920) affirmed the same thing for mesenchyme in general.

An abundance of mesenchyme at this stage of differentiation is available for examination in the embryos studied. Phases of the differentiation of cartilage, fiber bone, skeletal and smooth muscle have

tion of the mesenchyme involved in capillary formation leaves this cell not only as one producing reticulum, but with tremendous possibilities of differentiation, a very simple one being into fibrous tissue.

Evidence of the transformation of reticulin into collagen has been set forth by Rössle and Yoshida <sup>16</sup> (1909) in their studies of the reticulum of lymph nodes in normal and pathological conditions. Rusakoff <sup>17</sup> (1909) likewise found little distinction in the chemical nature of reticulin and collagen. Miller <sup>18</sup> (1927) in studies of the reticulum in tuberculosis, although he did not assign a specific origin for reticulum, considered it a precollagenous type of connective tissue and observed this transformation in the healing process of tuberculosis. Foot, <sup>19</sup> in his excellent critical review of the endothelial phagocyte, gives an extensive bibliography touching many phases of the problem in hand and cites other evidence, including his own, dealing with the transformation of reticulin into collagen. The writer too, has observed the apparent transformation of reticulum fibers into fibers staining as collagen, under a variety of circumstances. Although a consideration of the rôle of reticulum in tuberculosis, as revealed by the present method of staining, will be the subject of a future report, some of the most convincing evidence of this change has been observed in this disease. In Fig. 6 is shown a tubercle in the liver in a case of miliary tuberculosis. At the periphery of the tubercle, it may be seen that the reticulum fibers lining the liver sinusoids have become thickened; with this thickening the fibers take the golden brown color of collagen in silver preparations and the rose red in gold toned sections. Other evidences of transformation of reticulin into collagen have been observed in tuberculosis.

#### RETICULUM IN THE LIVER, SPLEEN AND LYMPH NODES

The liver, spleen and lymph nodes form a special phase of the reticulum problem. Each has received considerable attention in the literature and the writer can only touch upon his findings. In the liver Kupffer <sup>20</sup> (1876) described the reticulum under the name of *Gitterfasern*. In well prepared sections, the reticulum fibrils of the liver are striking objects. Deep brown to jet black fibers lie against the columns of liver cells forming the immediate lining of the sinusoidal vascular channels. It is not uncommon to see fibers cross over from one side to the other. The fibers are frequently seen in

Intimately associated with the problem under consideration is the question of the derivation of capillaries. The weight of evidence favors the concept that capillaries are formed *in situ* as a direct differentiation of the mesenchyme. Among those favoring this point of view Pulford<sup>10</sup> cites Reichert, Goethe, Felix, Rückert, Mollier, Maximow and Bonnet. In this country McClure,<sup>11, 12</sup> Reagan<sup>13, 14</sup> and Stockard<sup>15</sup> have contributed convincing evidence supporting the local origin of capillary endothelium. Hueck recently lends further support to this concept. The writer's study of pig embryo sections has convinced him that capillary endothelium is formed *in situ* by a very simple modification of the mesenchyme. This transformation is probably the simplest differentiation involved in the mesenchyme. The delicate fibers of this tissue are slightly rearranged and come to encircle the tube-like cleft in the mesenchyme. The new formed capillary is only readily recognized by its content of red blood cells. The cells lining it resemble those of the undifferentiated mesenchyme, and as a matter of fact, delicate fibrils can be traced from the lining cell into the undifferentiated mesenchyme. The cells lining the capillary possess the same delicate fibrillar processes as the mesenchyme. The fibrils stain darker, black or almost so. Reference to Fig. 4 and Fig. 2 will show this clearly. In Fig. 2 the small capillary in the mesenchyme underlying the corium shows distinctly the reticulum fibrils forming its wall. The nuclei in the primitive capillary wall are morphologically identical with those of the surrounding mesenchyme, and reticulum fibers extending from these nuclei not only surround the vascular channel but anastomose with the fibers of the surrounding mesenchymal cells. A capillary with the same cytological details remains in the fibrous corium above. Fig. 5 is a photomicrograph of subepithelial tissue showing an early phase in transformation of mesenchyme into fibrous tissue, containing two capillaries in which the fibered lining cells are recognizable.

In all capillary endothelium studied, both embryonic and adult, including practically all organs and tissues, reticulum fibrils have been demonstrable, thus confirming and extending the findings of Corner.

Demonstration of the delicate fibrils in mesenchyme serves to account for the reticulum fibrils in various tumors of mesenchymal origin as observed by Mallory and Parker. The very slight modifica-

or the ability of the writer to trace the capillary formation in all organs, but an outline of two general concepts, it is believed, will explain the principles of capillary formation. In tissue directly differentiated in the mesenchyme, as the heart and skeletal muscle, undifferentiated mesenchymal cells remain lying against the differentiated structures, and being applied to adjacent fibers, a potential if not actual channel lined by fibrillated mesenchymal, now endothelial, cells exists. In epithelial structures such as the lung, thyroid and hypophysis, the epithelial cells grow into the mesenchyme leaving mesenchymal cells applied against the epithelial cell columns or alveoli, forming the lining of the finer vascular spaces. As previously indicated, Corner has shown reticulum fibrils in the capillary endothelium of the corpus luteum, adrenal, hypophysis, thyroid and kidney. Except the corpus luteum, which has not been studied, the writer has been able to confirm these findings. Fig. 9 shows an area of lesser reaction in the lung in a case of miliary tuberculosis. Here may be seen cells with distinct black fibers forming the immediate lining of capillary vascular channels. Fig. 10 shows the continuity of reticulum fibers and endothelial cells in the medulla of the adrenal. The same findings hold for the cortical endothelium shown in Fig. 11.

Fig. 12 shows the black fiber lining of the capillary endothelium of the anterior lobe of the hypophysis.

Fig. 13 shows the same for the thyroid. A single cell layer existing between the vascular channel and the epithelium precludes the possibility of this being a fibroblastic layer. The reticulum fibers are flattened down, due to the pressure of the contained colloid in the alveoli.

Fig. 14 shows two small capillaries in the brain, one containing red cells, the other smaller and empty. Here in vessels a single cell layer in thickness, are clearly shown reticulum fibrils in the endothelium. With complete impregnation of the fiber substance of the capillaries, combined with adequate counterstaining a more complete concept of the finer vascular channels has been achieved which is set forth below.

*The Finer Capillary Bed:* Fig. 15 shows a congested area in the cortex of the kidney of a child. Examination of this figure, which is a faithful reproduction of an actual microscopic field, readily shows the following: (1) continuity of delicate black fibers (reticulum

intimate association with, and radiating from cell nuclei projecting into the lumen of the sinusoid, that are considered to be the Kupffer cells. Fig. 6 illustrating the transformation of reticulin into collagen about a tubercle also illustrates the general cytology of reticulum in the liver.

From incomplete studies thus far made the writer feels that the pathology and pathological physiology of the spleen is to a large extent bound up with the problem of the fiber substance of the spleen. In spleens that can be considered essentially normal, reticulum fibrils are readily demonstrated in the capillary endothelium and in a portion of the intersinusoidal or so-called reticulum cells. Normally these are delicate fibrils and not over abundant. In Banti's splenomegaly the reticulum fibrils of the intersinusoidal cells are remarkably increased in number, coarsened, and many fibers transformed into a collagen-reacting substance, giving a golden brown silver reaction, and a rose red in toned sections. Fig. 7 illustrates such a spleen. In lymph nodes, reticulum is again demonstrable in the capillary endothelium. Rössle and Yoshida found so-called reticulum, stainable by the Bielschowsky-Maresch method, in the lymph sinuses, the lymphoid tissue and the capillaries. It was considered as a precollagenous substance and transitions into collagen were noted by them. They found no distinction between the resting reticulum cells and the so-called endothelium of the lymphoid sinuses. The writer's findings are in accord with the above and with the careful studies of Downey who derived the reticulum cells supporting the pulp cords and nodules, as well as the cells lining the lymphoid sinuses, directly from the mesenchyme, and demonstrated the capacity of both to produce reticulum fibrils. Fig. 8 shows clearly reticulum fibers extending from the endothelial cells of the lymphoid sinuses. Morphologically similar cells form the supportive stroma of the lymph cords. In other areas reticulum fibers are seen in the capillary endothelium.

#### UNIVERSAL OCCURRENCE OF RETICULUM FIBERS IN CAPILLARY ENDOTHELIUM

As examination of the accompanying photomicrographs and drawings indicates, fiber substance, reticulum, is demonstrable in all capillary endothelium. It would be beyond the scope of this paper



lation is generally conceded for these organs, they were omitted from detailed consideration. A glance at the section of thyroid will confirm the same concept for its circuit.

These preliminary studies indicate that the capillary bed is tremendously greater than generally conceived. The unopened bed is outlined by reticulum fibrils of endothelial cells. Such lines of communication probably ordinarily serve as channels for the conveyance of non-corpuscular elements of the blood, and, it is believed, may open under effective stimulus into channels of sufficient size to carry corpuscles. The reticulum fibers are in intimate contact with the parenchymatous cells of the organ and are, in fact, identified with the basement membrane in the kidney, pancreas, adrenal and gastric mucosa. Corner identified these fibers with the basement membrane in the kidney. It will be seen, then, that the capillary circuit is of an absolute character.

The concept is set forth as a morphological basis for the well known physiological capacity, as demonstrated by Krogh<sup>21</sup> and others, of the capillary bed to increase suddenly in extent.

The capillary bed is conceived as one of an absolute character, *i. e.*, one of the highest efficiency, touching the surface of all parenchymatous cells. A morphological background is given for the well known physiological capacity of the capillary bed alternately to open and rest and, under effective stimulus, to increase greatly in extent.

## SUMMARY AND CONCLUSIONS

A new method of metallic impregnation is detailed which yields complete impregnation of mesenchymal, reticulum and collagen fibrils. An adequate polychrome counterstain may be superimposed upon the impregnated tissues. The mesenchymal cells possess a rich delicate fibrillar cytoplasm; the fibrils are readily impregnated by the method employed. Morphological support is given for the generally accepted concept that capillaries are formed *in situ* by a direct differentiation of the mesenchyme. This differentiation of capillaries in the mesenchyme is of a very simple character. The capillary endothelial cell remains in the embryo as a fiber-producing cell and this property and capacity persists into the mature organism. Both reticulin and collagen are fiber products derived from a common fibrillar mother substance and are undoubtedly chemically similar.

fibers) with the endothelial cells in a perfectly simple one cell layer; (2) the identity of the basement membrane with the above described fibers; and (3) in oval, round or angular spaces are red blood cells, *i. e.*, capillaries as seen in ordinary sections; further (4) there are distinct lines of continuity clearly marked out by reticulum fibrils connecting the capillaries seen, *i. e.*, intercapillary channels potential and actual. It is the writer's concept that such channels serve normally as finer nutritive spaces and may, under stress of circumstances, open into spaces capable of carrying blood corpuscles. In other portions of the same slide, single red cells may be seen lying flattened in narrow clefts in the intercapillary reticulum lines. Fig. 16 shows two such clefts in an intertubular reticulum line, each of which contains red blood cells.

The same concept is also well illustrated in a study of the pancreas in Fig. 17. Here again continuity of reticulum fibrils and endothelial cells is seen; reticulum fibrils form the immediate lining of the capillary wall and, intercapillary reticulum-lined spaces are readily demonstrable. The identity of the reticulum fibrils and the basement membrane is again shown. The capillaries of the islets of Langerhans (not illustrated) also show a fibered cytoplasm and in favorable sections surface views may be seen. Here, focusing high at the surface of the capillary, very delicate slightly undulating black fibrils paralleling the longitudinal axis of the vessels are seen. At the lateral borders they are viewed several layers thick and show as darker lines.

The gastric mucosa (Fig. 18) illustrates the same principles outlined for the kidney and pancreas.

It is in connection with study of the heart muscle that the most striking support of the concept of finer capillary radicles is achieved. Fig. 19 shows an area of heart muscle in tangential section. Here a number of reticulum-lined capillaries with contained red blood cells are shown in cross-section and the reticulum-lined, intercommunicating spaces readily seen. Continuity of reticulum fibrils and the capillary lining cells is demonstrated. Selecting an edematous and congested heart muscle, Fig. 20, we see many of the potential spaces opened, in fact a veritable reticulum mesh containing red blood cells at various points. The photograph lacks sufficient detail to show these clearly. The same principles apply to the circulation in the adrenal and hypophysis, but inasmuch as a sinusoidal type of circu-

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Reticulum fibers are demonstrated in capillaries in a wide variety of tissues, sufficiently wide to justify the concept that they are of universal occurrence in the capillary endothelium. Otherwise stated, reticulum may be identified as the fiber product of capillary endothelium. Similar fiber substance is present in the endothelial and reticulum cells of the lymph nodes. These cells, as the capillary endothelial cells, are little differentiated, direct descendants of the mesenchyme. Reticulum fibers are also present in the intersinusoidal or so-called reticulum cells of the splenic pulp and line the sinusoids of the liver. Reticulum fibers are a little changed descendant of the mesenchymal fibers.

Brief evidence is presented favoring the ability of reticulin to be transformed into collagen.

Reticulum is the most widespread and important supportive substance in the body. It is the scaffolding of cells and cell units. It serves the double purpose of microscopic cell support and the lining of capillary vascular channels.

By identifying reticulum with the capillary endothelium and obtaining sufficiently clear sections, the finer structure of the capillary bed is revealed. Reticulum fibers form the immediate lining of capillaries and minute reticulum-lined spaces are shown extending between and connecting the small capillaries as seen in ordinary sections. Such channels are considered to serve normally for the transfer of elements contained in the plasma of the blood and to be capable of enlarging or "opening up" under effective stimulus to a caliber sufficient to convey corpuscular elements. The endothelial reticulum is identified with the basement membrane in the kidney, pancreas, adrenal and gastric mucosa; this probably applies to basement membranes in general.

The author wishes to thank Dr. G. Y. Rush and Dr. C. L. Connor for stimulation and assistance received in the conduct of this study.

## DESCRIPTION OF PLATES

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### PLATE 108

- FIG. 1. Section of skin and subcutaneous tissue, 7.5 cm. pig embryo. Beneath the epidermis the mesenchyme is undergoing a transformation into fibrous tissue. The fibers have become rearranged longitudinally and now take the golden brown tone of collagen. The undifferentiated mesenchymal mesh is seen in the lower portion of the section. Stain: Method I.  $\times 80$ .
- FIG. 2. Detail drawing taken from same slide as Fig. 1, showing the rearrangement of fibers in formation of the corium. The coarser longitudinal fibers take the rich golden brown tone of collagen. At the lower portion of the section is the looser fibrillar mesh of the mesenchyme. The wall of the small capillary in the mesenchyme is seen to be composed of cells, morphologically identical with those of the mesenchyme, the fibers of which not only form the wall of the capillary but anastomose freely with those of the surrounding mesenchyme. The fibers stain somewhat darker than those of the surrounding mesenchyme. If this capillary had "invaded" the mesenchyme it would have rearranged the fibers about it and certainly would not show delicate fibrils extending from its wall into the surrounding mesh.
- FIG. 3. Section of the lung, 11 cm. pig embryo. Lymphatic spaces appear as simple clefts in the mesenchyme in the interlobular septae. Stain: Method I. (See Fig. 4 for detail.)  $\times 80$ .

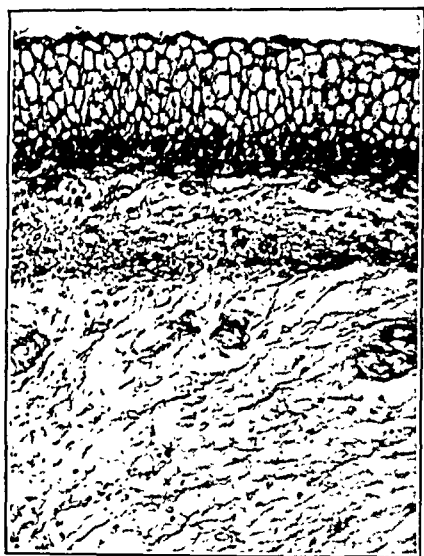
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PLATE 109

FIG. 4. Drawing from interlobular septum of 11 cm. pig embryo showing development of a small blood vessel in the mesenchyme. The anastomoses of the fibers forming the wall and of the intervening mesenchyme is clearly shown. The vessel is destined to be one of larger than capillary caliber. The arc at the right is a portion of the wall of a lymphatic cleft in the mesenchyme. Stain: Method I.

FIG. 5. Photomicrograph of two small capillaries in the corium in which the mesenchyme is being transformed into fibrous tissue. The fibers are assuming parallel arrangement and becoming coarser, and taking the deeper golden brown reaction of collagen. The fibers lining the two small capillaries are blacker than those of the surrounding mesenchyme. Stain: Method I.  $\times 520$ .

FIG. 6. Group of tubercles in the liver from a case of miliary tuberculosis. The general architecture of the reticulum lining the sinusoids is shown. At the periphery of the tubercles the sinusoidal reticulum is seen to be coarsened. In the original sections the color contrast is striking; the coarsened reticulum takes the golden tone of collagen, the reticulum away from the tubercles stains black. The coarsening of the reticulum is well shown in the sinusoids intervening between the tubercle at the right of the photograph and the group in the center. Stain: Method I.  $\times 80$ .

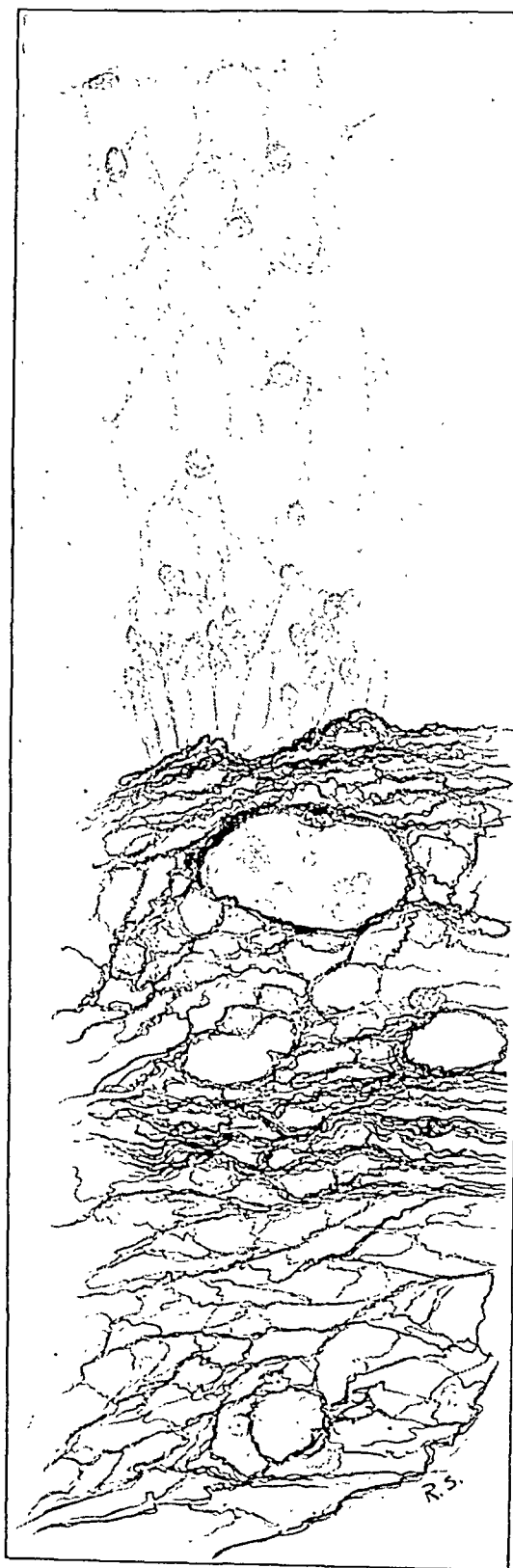


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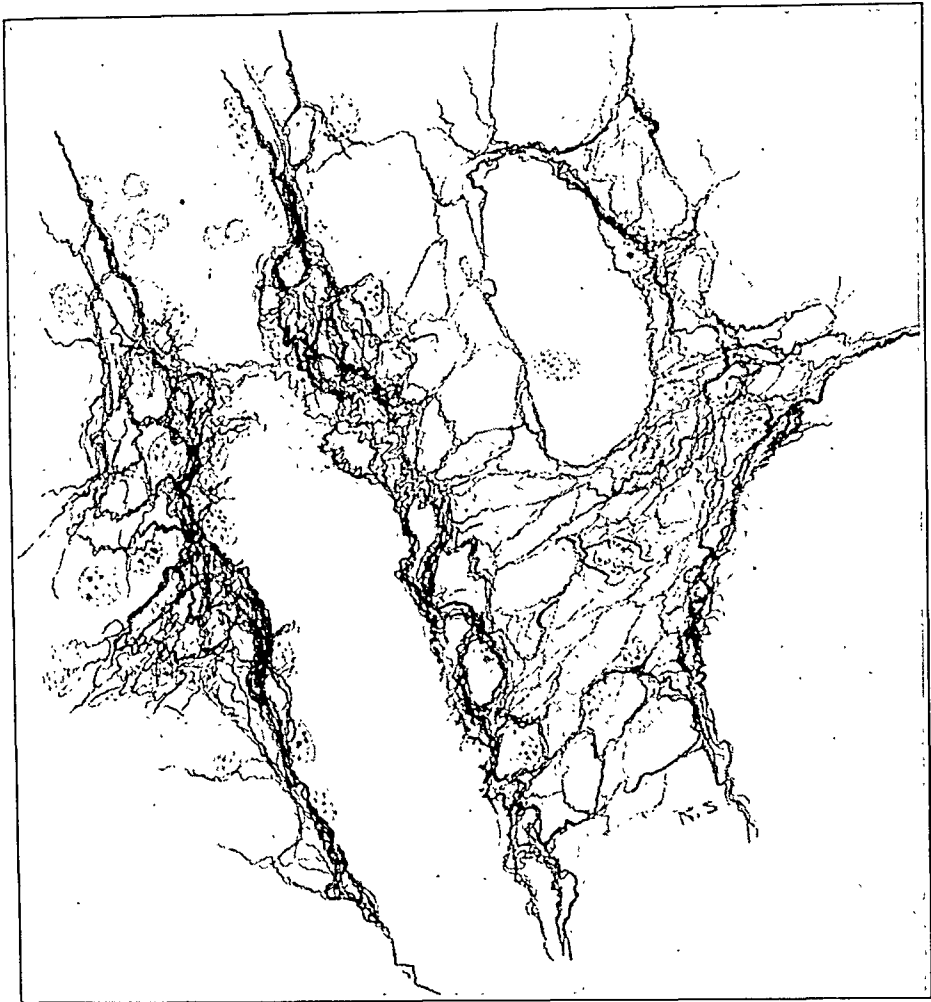
2

Reticulum



PLATE 110

- FIG. 7. Section of spleen in case of Banti's disease. A marked coarsening of the reticulum is seen. The coarse black areas shown in the photograph give the golden brown reaction of collagen. Those less coarse maintain the black reaction of reticulum. Stain: Method I.  $\times 150$ .
- FIG. 8. Section of essentially normal lymph node showing reticulum fibers continuous with the cells, lining and extending into the lymphoid sinus. Morphologically similar cells form the supportive stroma of the lymph cords. Other areas show reticulum fibers in the capillary endothelium. Stain: Method II.  $\times 520$ .
- FIG. 9. Section of lung in an area of lesser reaction in a case of miliary tuberculosis. Reticulum fibers extending from cell nuclei and forming the immediate lining of two capillaries is well shown. One runs horizontally at the top of the photograph. The other in the upper right hand corner is cut transversely. Proliferating large mononuclear cells without fibers are seen attached to the capillary wall and free in the alveolar space. Stain: Method II.  $\times 520$ .
- FIG. 10. The drawing taken from the medulla of the adrenal clearly shows the continuity of reticulum fibers with the endothelial cells lining the capillaries and extending as the basement membrane of the medullary cells. This figure also illustrates the concept of the closed capillary bed described in detail in the kidney, pancreas and heart muscle. A triangular cleft outlined by reticulum containing a single red blood cell is seen near the center of the field. At the upper left angle this cleft is closed but the reticulum fibers continue as the basement membrane.



4



Rinehart

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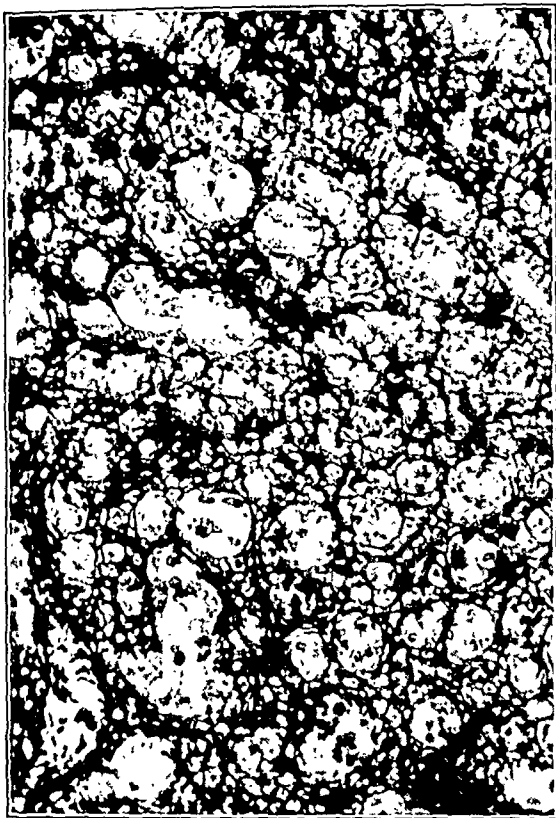


6

Reticulum

PLATE III

- FIG. 11. Photomicrograph of the adrenal cortex showing continuity of reticulum fibrils and endothelial cells lining the capillaries. Stain: Method I.  $\times 780$ .
- FIG. 12. Section of anterior lobe of the hypophysis showing the dense reticulum fibers lining the capillary spaces. Stain: Method I.  $\times 520$ .
- FIG. 13. Thyroid gland showing reticulum fibers lining open and closed capillary channels. A single cell layer existing between the vascular channel and the epithelium precludes the possibility of the reticulum being a fibroblastic layer. Reticulum fibers outline the open and potential capillary channels. Stain: Method I.  $\times 520$ .
- FIG. 14. Showing two small capillaries in the brain, one containing red cells, the other empty. Here in vessels, a single cell layer in thickness, are clearly shown reticulum fibrils forming the immediate vascular lining.



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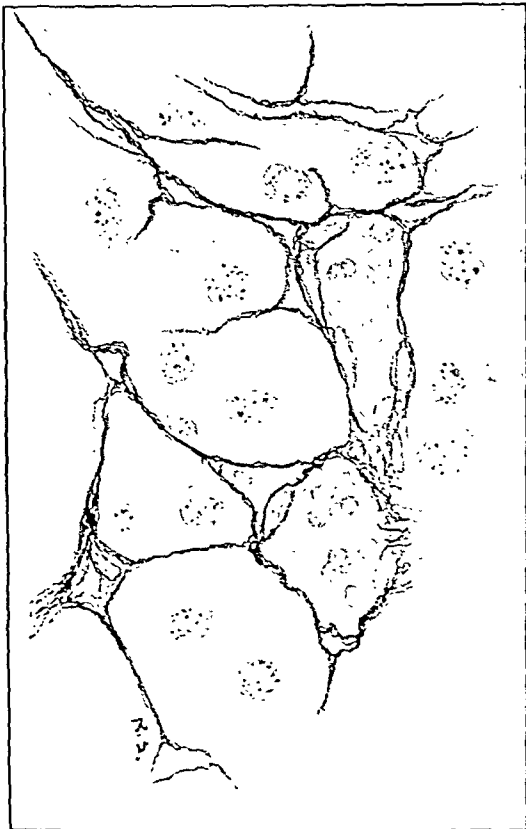


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Rinehart



10

Reticulum

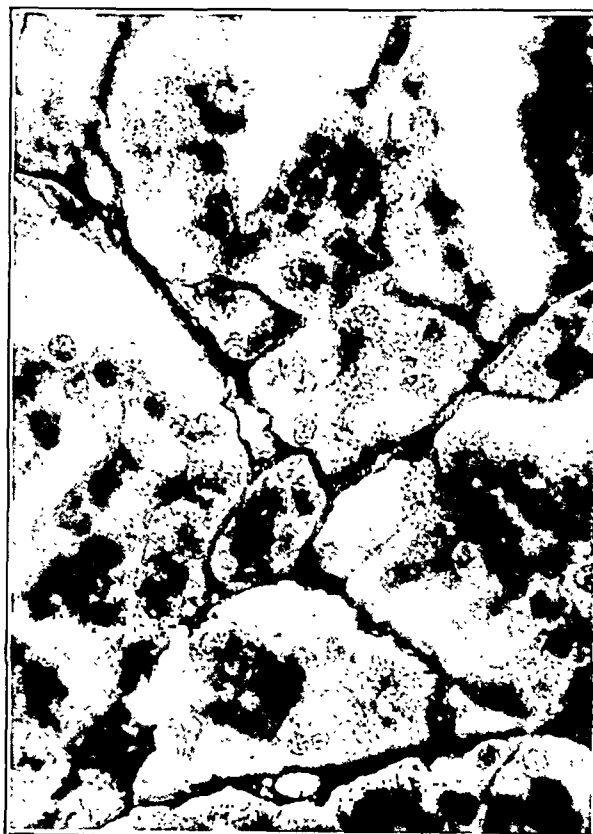
PLATE 112

FIG. 15. This drawing is taken from the kidney of a child. Here the following is shown: (1) continuity of delicate black fibers (reticulum fibers) with the endothelial cells in a perfectly simple one cell layer; (2) the identity of the basement membrane with the above described fibers; (3) oval, round and angular spaces, lined by reticulum fibers, containing red blood cells, capillaries as ordinarily seen, and, (4) connecting such spaces are fibers directly continuous with the capillary wall, forming lines of intercommunication between capillaries.

FIG. 16. Photomicrograph of the kidney illustrated in Fig. 20, showing two clefts in the reticulum "line" between two tubules. Red blood cells lie in each of these clefts. Stain: Method II.  $\times 520$ .



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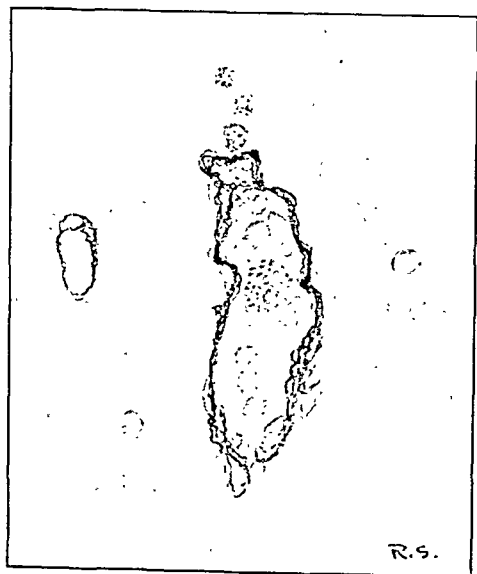


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Rinehart



14

Reticulum

PLATE 113

FIG. 17. Drawing from pancreas of child, showing reticulum-lined capillary spaces containing red blood cells. The identity of the reticulum and the basement membrane is seen. Most of the capillary spaces in this field show only the reticulum lining, without the cells of origin. Endothelial nuclei with reticulum fibers in continuity are seen, lying between adjacent pancreatic acini, conceived as the lining of empty, collapsed capillary spaces. Potential but closed spaces are seen extending between demonstrable capillaries. Such fine channels are considered to serve for finer nutritive interchanges, and to be capable of carrying corpuscular elements of the blood under effective stimulus. Stain: Method II.

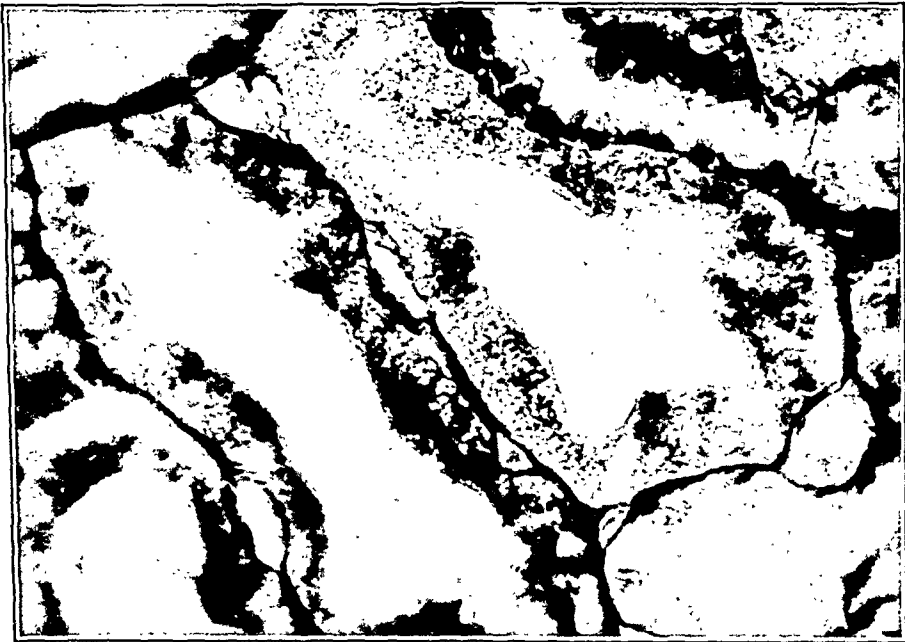
FIG. 18. Section of gastric mucosa, clearly showing reticulum fibers in the capillary endothelium and the identity of the latter with the basement membrane.

FIG. 19. Heart muscle in tangential section, showing continuity of reticulum fibrils with endothelial cells lining capillary spaces, and the continuation of these fibrils between capillaries forming minute passages for transfer of the blood plasma and conceived to be capable of "opening up" under effective stimulus for the conveyance of the corpuscular elements of the blood. Just above the center of the figure a single red blood cell is seen in a reticulum-lined endothelial cleft.

FIG. 20. Photomicrograph of a congested and edematous heart muscle, showing the reticulum-lined vascular spaces opened up. Although not showing clearly in the photograph, red blood cells are present at various points in the reticulum-lined capillary bed.



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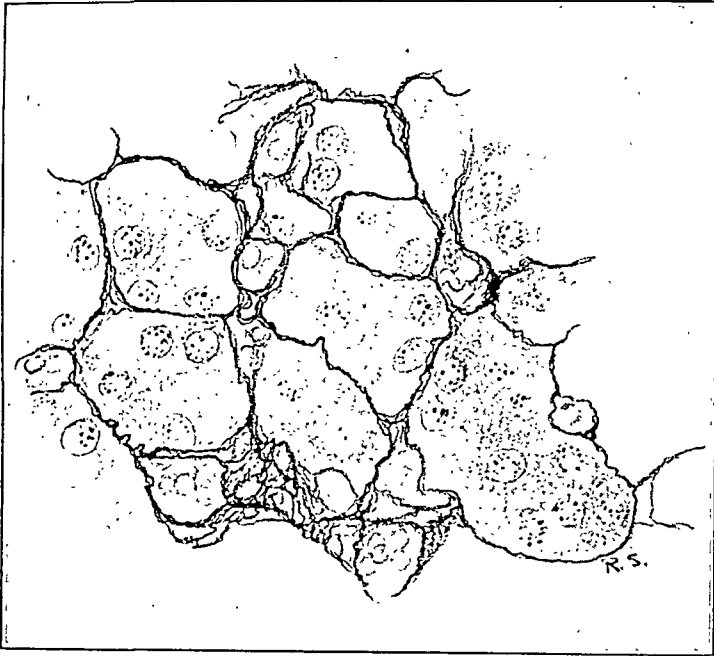
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Rinehart

Reticulum



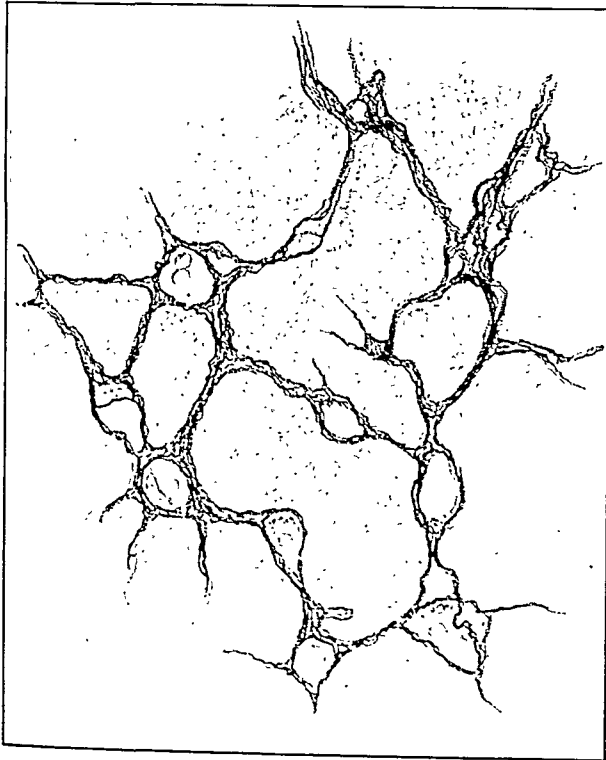




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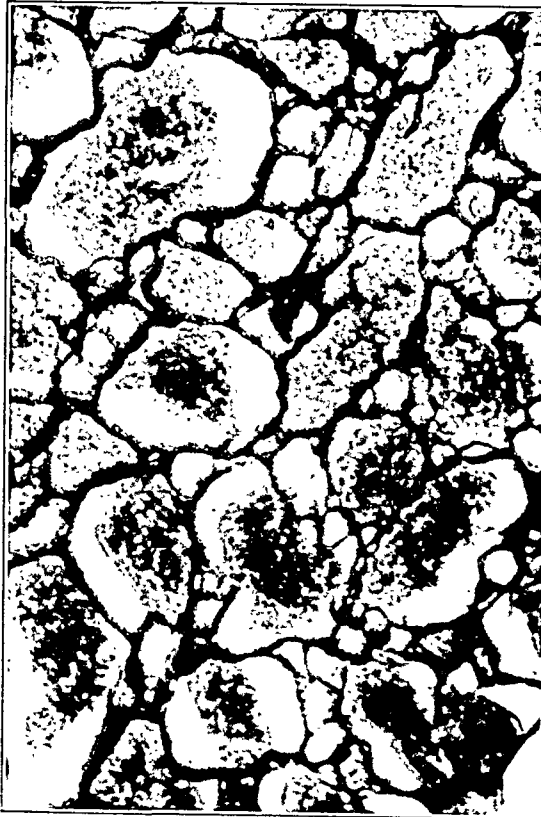


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19

Rinehart



20

Reticulum

Hoffheinz,<sup>3</sup> however, a year later expressed the opinion that the best results were obtained by the old method of quick fixation in hot formalin. He found that the dextrin solution caused sticky preparations which tended to form folds, and preferred the hematoxylin and eosin stain because it gave better color contrasts. Moreover, there was better preservation of the relationship of the various tissue structures. He concluded that the old method was a more dependable means of diagnosis, especially in obscure cases, justifying the five to ten minutes necessary for preparation.

Dudgeon and Patrick,<sup>4</sup> also in 1927, reported what they describe as a "wet-film method." The freshly-cut surface of the tissue is scraped and the juice thus obtained is spread on a slide and placed in Schaudinn's fluid for fixation. The films are then stained with haemalum and eosin. They allow eight to ten minutes for the complete process. In a series of 200 specimens they report successful results as compared with the control examination of sections prepared in paraffin by the usual technique.

MacCarthy for years has used frozen sections of fresh unfixed tissue, stained with Terry's modification of Unna's polychrome methylene blue. In 1928<sup>5</sup> he emphasized the importance of the cytological study of fresh unfixed material, and in a recent paper<sup>6</sup> states that the diagnosis of a malignant condition may be made from a single cell.

Taft and Ludlum<sup>7</sup> in a preliminary report have lately described a method for staining unfixed brain tissue with silver. The tissue is placed in argyrol, later washed in distilled water, and a film finally prepared.

In any method of quick diagnosis, the rapidity depends upon familiarity with a given procedure, the experience of the observer and the nature of the tissue. The morphological diagnosis of a tumor is occasionally very difficult whatever the method of preparation, and long and exhaustive study may be necessary before the final classification of the growth is possible.

*Technique:* The method, as stated, is that described in detail by Sabin<sup>8</sup> in her studies of living human blood cells. Its application to the more solid tissue of intracranial tumors is quite simple.

As soon as the tumor is exposed at operation, a fragment of it is routinely submitted for immediate examination. A minute portion is separated with dissecting instruments and placed on a glass slide.

## DIAGNOSIS OF INTRACRANIAL TUMORS BY SUPRAVITAL TECHNIQUE \*

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With increasing satisfaction and confidence we have come to rely upon the supravital technique, devised by Sabin for the examination of the blood, as our most favored method for the making of pathological diagnoses of intracranial tumors. In this procedure the tissue is neither fixed nor frozen, but is simply prepared as a fresh smear of the living cells, and with experience immediate diagnoses can be secured which are often more dependable than those based on the study of stained tissue sections.

Our attention was first drawn to the possibilities of this method by Dr. Lawrence Kubie, who three years ago spent several weeks in the neurosurgical laboratory studying the cytological elements, more particularly the ependymocytes, in the surgical specimens that happened to be supplied at that time. We have since then come to familiarize ourselves with the appearance of the tumors studied in this way and find that specimens, which have been prepared by the time-consuming methods in common use, fail in many instances to give the information we desire for purposes of classification. The method is of particular value in the differentiation of the gliomas into their various types, and believing that others will be interested in the subject, we propose to give a brief account of our experience with it.

Various methods for rapid microscopic diagnosis, particularly of malignant tumors elsewhere in the body, have been reported. Most of these, however, describe some process of fixation and the preparation of frozen sections. Hellwig<sup>1</sup> in 1926 advocated Wilson's method<sup>2</sup> of fixation in dextrin solution and staining of the sections with Unna's polychrome methylene blue, a procedure requiring about three minutes. They discovered that with the dextrin solution there was less shrinking and tearing of tissues than when formalin is used, and stated that individual cells were thereby more distinctly shown.

\* Received for publication June 9, 1930.

the central nervous system by this technique,<sup>9</sup> preparations of solid tissue tend to be uneven. Some areas are too dense, and others too thin. But there are many fields in which the relations of the different structures are well preserved, and in the thinner portions individual cells which have become separated may be studied in minute detail.

In every case for comparison a portion of the same tissue is fixed to be subsequently sectioned and stained by the usual methods.

*Advantages:* Possibly the greatest value of the method herein presented lies in the opportunity it affords for the study of cells which have not undergone changes due to fixation or cutting, but which are seen under the microscope in their entirety while they are still living. The usual shrinkage and distortion of the tissues is thus avoided. Under high magnification (oil immersion) single cells, such as astrocytes and oligodendroglia, may be beautifully defined with all their delicate processes which are rarely to be seen intact in fixed histological sections. After fixation the cytoplasm is oftentimes destroyed, or in other instances it is demonstrated only by the use of special stains. An illustration of this is given in the photomicrographs of a recent oligodendroglioma (*cf.* Figs. 9 and 10) in which it may be seen how distinctly the cytoplasm of each cell is outlined in the fresh smear, while in the fixed preparations it has become changed beyond recognition.

Mitotic figures, too, may be identified in various phases in the supravital preparations of some of these tumors.

Not only are individual cells clearly shown, but, as has been pointed out, the relationship of the various structures in the tumor as a whole is well preserved, such as the formation of whorls and masses of cells in a meningioma, or the radiation of the cells of an ependymoma about a vessel and its branches, or the palisade arrangement of cells so characteristic of an acoustic neurinoma.

In invasive types of brain tumors myelin sheaths are often identified as curious irregular strands with bulbous enlargements and terminations sharply outlined by what appear as two closely parallel lines which are extremely refractive (*cf.* Fig. 6).

Collagen, too, has a distinctive appearance because of the refractive quality of its bands of wavy fibers.

Of particular interest are the histiocytes, which are frequently associated with such tumors as the rapidly growing gliomas, men-

The slide may be previously prepared with an even film of neutral red dye, or a mixture of neutral red and Janus green, the exact directions for which are given by Sabin. We have found that satisfactory results are obtained when a clean slide is used and a drop of aqueous solution of neutral red dye (1:10,000 or more) added directly to the tissue.

A cover glass is immediately placed over the tissue, which is carefully spread by gentle pressure on the cover glass, the rubber end of a pencil being convenient for the purpose. One learns by experience how much pressure to apply in making the smear. Some tumors are so soft that they spread almost like fluid, and anything more than the lightest touch damages the cells. Other tissues are very firm, fibrous or even gritty with calcium, requiring greater effort in flattening them out for a satisfactory film.

The preparation is sealed at once with a mixture of vaseline and paraffin around the edge of the cover glass, and is then examined microscopically in a warm box.

The actual preparation of the smear takes less than a minute.

There are one or two precautions which may be particularly commented upon at this point. If too strong a solution of the dye is used, the nuclei will be stained diffusely — a sign that the cells have been injured. The edges of the preparations are usually overstained in this manner. While overstaining does not necessarily so obscure a microscopic picture as to make a diagnosis uncertain, it must be avoided if one is interested in studying the cytological elements of these tumors.

Furthermore, the importance of making the preparations as soon as possible after the tissue has been removed should be emphasized whether or not an immediate microscopic report is imperative. A diagnosis may be made in most cases even though the smears are prepared an hour or several hours later, provided that the tissue has not been allowed to dry. However, there is no question but that the cells undergo changes on long standing, especially if the tumor is of a soft consistency. They become swollen and vacuolated, the cytoplasm markedly granulated, and the nuclei show degenerative changes. When extreme vacuolization occurs, one vacuole may fill the whole cell. In some areas cytoplasmic boundaries become unrecognizable, so that there is only disintegrated material to be seen.

As Kubie has pointed out in a study of the perivascular tissues of

knowledge of the cytological characteristics of a tumor is therefore of great value in teaching him to associate the histological picture with the gross appearance of the lesion. Furthermore it gives him an immediate indication of the prognosis so that he may modify his measures accordingly and determine what particular operative procedure to pursue and how radical or otherwise an extirpation of the lesion is desirable. Moreover if the growth is of a type benefited by roentgen-ray therapy, the latter may be instituted without delay.

This of course is less important with the tumors whose gross characteristics are better known, such as the acoustic neurinomas and the meningiomas, but even here operative difficulties of diagnosis may be encountered, a tumor, which was adherent to the dura proving histologically to be a carcinoma, whereas it was assumed to be a meningioma during the course of the operation.

*Description of Tumors:* A certain familiarity with the appearance of the different intracranial tumors in supravital preparations as contrasted with fixed preparations must be acquired before the various types of lesions can be distinguished with accuracy. A description of the microscopic appearance of the more common tumors when examined by supravital technique will therefore be given.

*Gliomas:* The gliomas represent over forty per cent of all intracranial tumors. The immediate differential diagnosis of the various types, as has been said, is important in indicating the prognosis and in determining the method of operative procedure. The three largest groups in the classified series are the astrocytomas, the glioblastomas, and the medulloblastomas, and they may accordingly be considered in the order of their frequency as follows.

*Astrocytoma Fibrillare:* When the tissue is quite tough and unyielding, and indeed it may contain calcium, the usual picture under the microscope is of a very dense network of neuroglial fibrillae which are rarely stained but appear as closely crisscrossing refractive processes, among which there are scattered nuclei. It is usually in the less dense areas along the margins of the preparation that the cytoplasm of the cells is most distinctly seen, and here one may find unmistakable astrocytes with their long processes extending in star-like fashion from the cell body, one of which not infrequently may be traced to a neighboring vessel.

Occasionally these tumors are soft in composition, the tissue spreading very easily. We have noticed especially in cases of such

ingiomas, and adenomas, and which are believed by Carrel<sup>10</sup> to influence growing cells by aiding in their nutrition. In these supravital preparations they may be observed during their activity to phagocytize the neutral red dye and store it in the digestive vacuoles within their cytoplasm. These vacuoles appear as round, highly refractive globules, which may increase greatly in size as they become filled with the inclusions of the dye. Clasmatocytes may be present in extraordinary numbers in a rapidly growing tumor like a medulloblastoma, showing evidence of great activity, or they may be scattered here and there, as in a pituitary adenoma, moving rather lazily among the tumor cells.

In some of the tumors there are other cells which contain highly refractive vacuoles in their cytoplasm, but which are non-motile and do not ingest the dye. These vacuoles are an evidence of cell degeneration. Such vacuolated cells are commonly found in acoustic neurinomas which are undergoing fatty degeneration, or occasionally in a meningioma they are conspicuous among masses of the tumor cells or within the whorls.

*Diagnosis:* In many cases the precise nature of the lesion is quite apparent to the operator from its gross appearance and location, as is usually true for example of such growths as the meningiomas. Under these circumstances the microscopic diagnosis is merely corroborative. On the other hand, there may be some uncertainty in the surgeon's mind as to whether, for example, an atypical soft vascular tumor exposed in the region of the sella turcica is a suprasellar meningioma or a pituitary adenoma, or whether a suspicious nodule apart from the main mass of tumor represents an implantation or metastasis, or whether a necrotic area consists possibly of degenerated brain in the neighborhood of the growth or actually is tumor.

Possibly the most important immediate differentiation that is needed is between the various types of gliomas, and although increased familiarity on the surgeon's part enables him to distinguish many of these tumors by their gross characteristics, this is only possible when he has become thoroughly familiar with its histological appearance. If the surgeon must wait for a week or two, or even for a few days before he can get a clear idea of the microscopic structure of the tumor he has seen at operation, his recollection of the gross appearance has necessarily become obscured. The immediate



refractive globules which are often large. Active clasmatocytes ingesting the neutral red dye are also present (Fig. 5). Among them the nuclei of the tumor cells may be seen, and usually one finds scattered myelinated nerve fibers indicating the invasive nature of the growth.

It may be pointed out that occasionally a bit of tissue is submitted for diagnosis from the brain in the neighborhood of a tumor. Under these circumstances the myelinated nerve fibers (Fig. 6) present an unmistakable picture. At other times the tissue may come from the margin of the growth, and examination disclose neuroglial fibrillae and possibly astrocytes, which, however, represent a gliosis rather than the real tumor, so that a more representative piece of tissue is necessary in order not to confuse the diagnosis with that of a fibrillary astrocytoma.

*Medulloblastoma:* In Figs. 7 and 8 are shown for comparison the preparation by supravital technique and the preparation of the same tumor which had been fixed, cut and stained by the usual method, the nuclei being greatly shrunken and the cytoplasm practically indiscernible. These tumors are generally soft and should be spread gently. Examination by supravital technique shows a most distinctive picture of a rapidly growing exceedingly cellular tumor, composed of masses of small round cells with round or oval nuclei containing a fair amount of chromatin. The predominating type of cell found in the fresh preparations as seen under oil immersion (Fig. 7) is round rather than carrot-shaped. The cytoplasm is pale in contrast to the nucleus, which sometimes may be a little eccentric, but the cytoplasmic boundary is very well defined, as is evident in the photomicrograph. Mitoses in various phases are easily recognized and may be numerous with distinct chromosomes, as was true of this particular tumor from the roof of the fourth ventricle in a child. Occasionally spongioblasts and neuroblasts may be identified in these tumors, but in this case they were not present.

Hordes of clasmatocytes are often present in medulloblastomas and may be seen actively taking up the dye so that they stand out conspicuously among the tumor cells. We have already referred to Carrel's conclusions regarding their influence on the nutrition of growing cells, and in this particular tumor, which is obviously a very rapidly growing one, they were present in extraordinary numbers none happening to show in the photomicrograph.

fibrillary astrocytomas removed from the cerebellum in young children that one is likely to find instead of a dense feltwork of fibrillae an abundance of well preserved astrocytes throughout the preparation. The cells may be quite small with a few processes forming a delicate fibrillary meshwork. In Fig. 1 an astrocyte of this type is shown and particular attention may be called to the distinctness of the nucleus and chromatin particles, and the granules in the cytoplasm.

*Astrocytoma Protoplasmaticum:* The protoplasmic astrocytomas are usually soft. The most pronounced difference from the fibrillary type when examined microscopically is the general absence of neuroglial fibrillae. Furthermore they tend to undergo degenerative changes and become invasive, and it is not unusual to see among the fairly large oval nuclei of the tumor numerous degenerated cells packed with vacuoles, and clasmatocytes which have taken up the neutral red dye. Nerve fibers identified by their refractive myelin sheaths may also be found here and there. In this respect they are similar to the glioblastomas.

If the specimen is not too degenerated, however, the cytoplasm as well as the nucleus may be discerned, and in Fig. 2 the soft branching processes of a typical astrocyte may be seen extending out in various directions for a considerable distance. The nucleus appears darker in the photomicrograph than it should because of the attempt to reproduce satisfactorily the delicately outlined processes which were only slightly stained.

How much more informing the supravital preparation may be when contrasted with the Zenker-fixed specimen from the same tumor stained with phosphotungstic acid hematoxylin is evident from Figs. 3 and 4, which have been taken at a low magnification to show the general architecture rather than the cell type of the tumor.

*Glioblastoma Multiforme:* These rapidly growing tumors are composed of cells of various size and shape, including spongioblasts of all forms, astrocytes, round and spindle-shaped cells, which in a favorable piece of tissue are easily identified in the fresh smear. Multinucleated cells are present and mitotic figures may be demonstrated. However, many of these lesions are degenerated, and if the tissue examined is from a necrotic area such cells are not well shown, but instead the fields are occupied by great numbers of vacuolated cells which may be huge in size, filled with characteristic greenish

appeared in the fresh preparation. They lie more or less parallel to one another, this arrangement having been quite consistent throughout all fields.

*Pituitary Adenomas:* The differential diagnosis between the chromophobe and chromophile types of pituitary adenomas is possible on examination of the supravital preparations. The chromophobe lesions are composed of masses or cords of epithelial cells which are round or polygonal in form with round or oval nuclei containing a variable amount of chromatin and usually well marked nucleoli (Fig. 13). The cytoplasm is very finely granular. Clasmotocytes are commonly found though they are generally not numerous and may be comparatively inactive. It may be noted that in the case of the youngest patient in the series with chromophobe adenoma, a girl who was first operated upon at the age of ten years, mitoses were observed in the fresh preparation of the tumor at a second operation five years later.

In the chromophile type the alpha granules which in fixed preparations are brought out best by special stains, such as Bailey's ethyl violet-orange G, are readily seen in the fresh smears as rather coarse granules most marked at the periphery of the cytoplasm. Multinucleated cells may be numerous and crescent-shaped cells are also found. There is considerable variation in the size of the cells. A rather striking picture was observed in a recent case of "fugitive acromegaly" upon examination of the fresh smear. Everywhere among other comparatively pale, delicately granular chromophobe cells there were great numbers of enormous cells which were especially conspicuous in appearance because of the very granular cytoplasm which stained deeply with the neutral red dye (Fig. 14). The nuclei were round with a prominent nucleolus, and some of the cells contained two nuclei.

*Meningiomas:* These tumors as a rule are unmistakable on examination by supravital technique. They vary in consistency from very soft to very firm lesions. One sees masses of cells with large nicely outlined oval, round or elongated nuclei which have a singularly typical appearance. Nucleoli are usually prominent. Whorl formation is well preserved in the fresh smears as may be seen in the photomicrograph which is taken at the same magnification as that of the fixed preparation of the same tumor (Figs. 15 and 16).

Even when calcium is present a smear satisfactory for diagnosis

*Oligodendroglioma:* Here again, in Figs. 9 and 10 the supravital preparation is shown in contrast with the fixed preparation of the same tumor stained by ordinary methods. These tumors are rare, representing only one per cent of the gliomas, so that only a few have been available for study by the supravital method of examination. When seen in the fresh smears they present a most interesting picture. Whereas in the fixed preparation (Fig. 10) the round though shrunken nuclei are seen, in no field of any section was the cytoplasm recognizable, though it may be noted that in some fixed preparations of similar lesions the nuclei may be surrounded by a halo. This may be contrasted with the fresh smear, which shows a typical field of the same growth at the same magnification (Fig. 9). The nuclei are spherical and unshrunken, containing quite abundant chromatin, and are surrounded by a pale cytoplasm which is very distinctly outlined. One may see in the photomicrograph how cellular the tumor is, but unlike the medulloblastomas, no mitoses are present. They are slowly growing tumors and almost invariably tend to undergo partial calcification.

The oligodendroglia are thought to develop from indifferent cells of ectodermal origin which may also differentiate into fibrillary astrocytomas. In a recent case of fibrillary astrocytoma of the cerebellum in a child of ten years, typical oligodendroglial cells were found in the supravital preparations of the tumor. In Fig. 11 two of these cells are shown with the slender processes characterized by swellings along their course and at their terminations. In fixed preparations the processes of these cells are not stained by usual methods, though they may be brought out by special silver stains as recently reported by Bailey and Bucy.<sup>11</sup>

*Other Types of Gliomas:* Of the remaining types of gliomas which form a smaller percentage of the classified group, one or two may be briefly referred to. There have been a few examples of ependymoma studied by this technique. They are highly cellular lesions and in the fresh smears the radiation of the cells around the vessels has been observed to particular advantage under low power, while under oil immersion extensions of the cells may be traced to the vessel walls. Blepharoplasten have not been distinguished in the tumors so far available for supravital study.

There have been two recent examples of spongioblastoma unipolare et bipolare. In Fig. 12 some of the individual cells are shown as they

with its gross appearance at the operating table, but a permanent photographic record of the fresh preparations can be made for comparison with the permanent section of the fixed tissue.

The supravital method makes it possible for the examiner to see the cells with their cytoplasm and processes intact and gives pictures which are wholly unfamiliar to those who have only studied these cells in fixed sections.

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## DESCRIPTION OF PLATES

### PLATE 114

- FIG. 1. Supravital preparation showing a single fibrillary astrocyte. Note the distinctness of the granules in the cytoplasm.  $\times 850$ .
- FIG. 2. Supravital preparation showing a single protoplasmic astrocyte. Note the numerous soft branching processes.  $\times 850$ .
- FIG. 3. Supravital preparation of an astrocytoma. For comparison with Fig. 4.  $\times 300$ .
- FIG. 4. Zenker-fixed preparation of same tumor as in Fig. 3 (phosphotungstic acid hematoxylin stain).  $\times 300$ .

may be obtained. The psammoma bodies under low power are conspicuous round refractive bodies, and when examined under oil immersion nuclei within some of these concentric concretions may be identified.

Occasionally there is evidence of degeneration, and in one of our recent cases the cytoplasm of the tumor cells within and outside of the whorls was filled with very fine greenish globules.

Clasmatocytes are associated with some of the meningiomas, as has been pointed out, and they may be observed ingesting the neutral red dye.

Although in most cases the gross appearance of a meningioma leaves no doubt in the surgeon's mind as to its histological nature, in rare instances the gross features may be misleading. On a recent occasion, for example, a tumor which was adherent to the dura and was regarded as a meningioma proved by supravital preparation to be a carcinoma.

*Acoustic Neurinomas:* The acoustic neurinomas are usually firm in composition. If the tissue is well preserved and cellular, the fresh smear shows oval or elongated nuclei running along in fibrillary strands in the palisade and occasionally whorl-like arrangement which is typical of these growths. The cytoplasm of the cells in such fields is ordinarily not well defined, but the general architecture of the tumor is unmistakable.

Frequently the tissue submitted has a yellowish fatty appearance, and when examined by supravital technique it shows the characteristic arrangement mentioned above, but the cytoplasm is more distinct because it is packed with fine greenish refractive vacuoles. The cells are commonly elongated and spindle-shaped, with fibrillary extensions at their extremities. When the tumor is extremely degenerated, however, many of the cells show only the nuclei, with globules of fat trailing along the fibrillary material present.

### CONCLUSIONS

The supravital technique has been adopted as the most favored routine method of diagnosing and classifying tumors of the central nervous system, it being of particular value in the cytological differentiation of the various types of gliomata.

Not only can an immediate diagnosis be given to the surgeon so that he may learn to associate the microscopic type of the lesion

PLATE 115

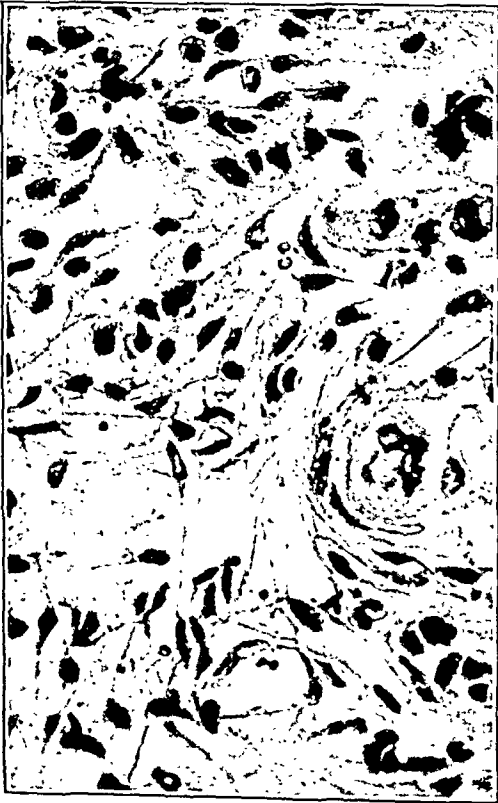
- FIG. 5. Supravital preparation showing vacuolated cells in a rapidly growing glioma. The upper cell is inactive, while two histiocytes below have ingested particles of neutral red dye.  $\times 600$ .
- FIG. 6. Supravital preparation of fragment of brain to illustrate the typical appearance of myelinated nerve fibers. They are highly refractive and are often found among the cells of an invasive tumor.  $\times 600$ .
- FIG. 7. Supravital preparation of a medulloblastoma showing clearly defined cytoplasm (arrows) and uniformly round shape of cells. Two mitotic figures are included in the field.  $\times 850$ .
- FIG. 8. Formalin-fixed preparation of same tumor as in Fig. 7 (hematoxylin-eosin stain). The cytoplasm has been almost wholly lost and the nuclei are greatly shrunken.  $\times 850$ .



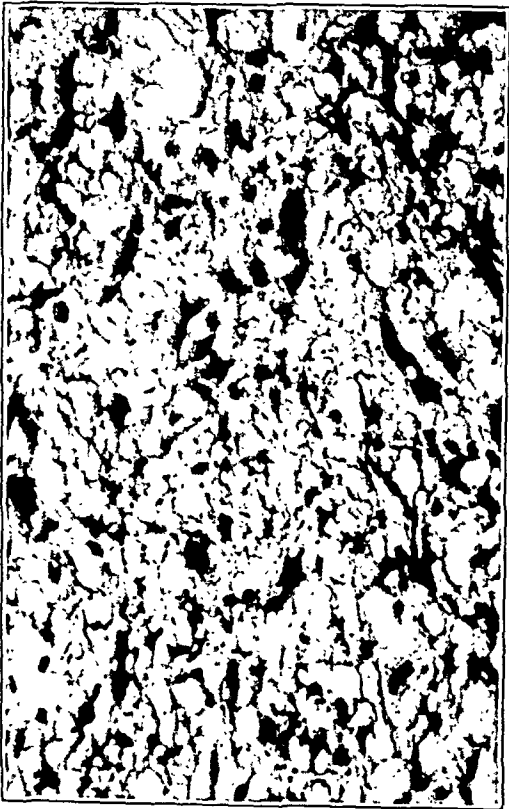
1



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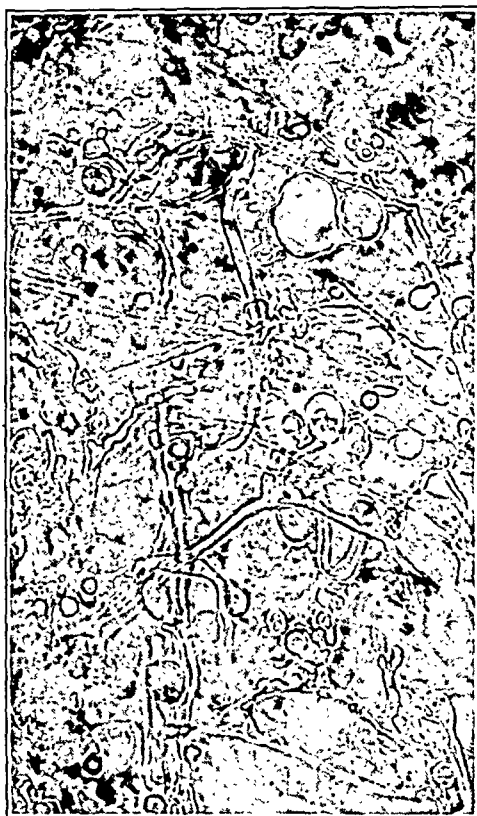


PLATE 116

- FIG. 9. Supravital preparation of an oligodendroglioma to show the sharply outlined cytoplasm of the cells and their spherical nuclei. For comparison with Fig. 10.  $\times 850$ .
- FIG. 10. Zenker-fixed preparation of same tumor as in Fig. 9 (eosin-methylene blue). The cytoplasm was not recognizable in any field of several sections studied. The nuclei are much shrunken.  $\times 850$ .
- FIG. 11. Supravital preparation showing two isolated oligodendroglial cells from a midline cerebellar fibrillary astrocytoma, with typical swellings along their processes.  $\times 850$ .
- FIG. 12. Supravital preparation of a spongioblastoma unipolare et bipolare, showing several of the individual cells in characteristic parallel arrangement.  $\times 850$ .



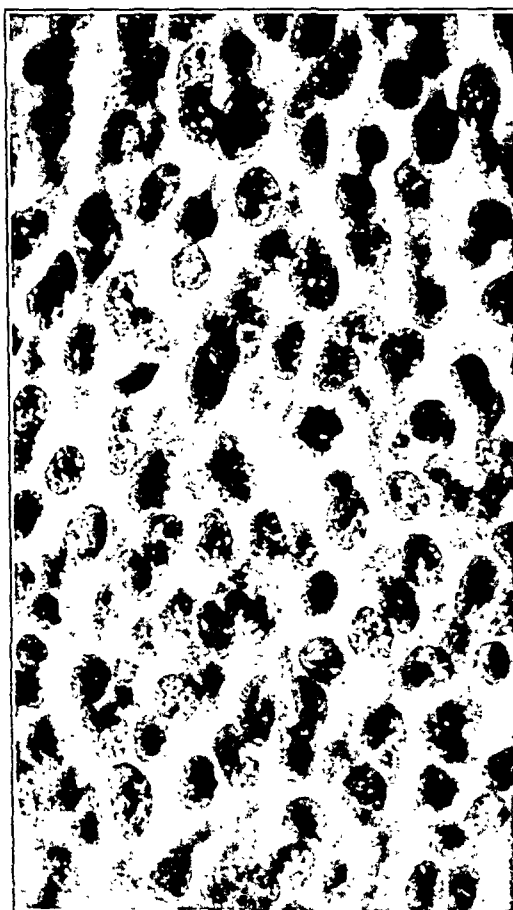
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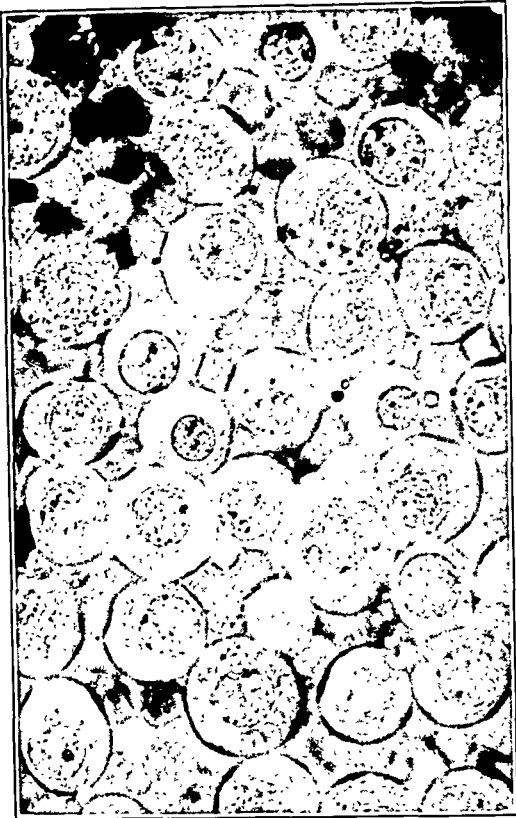
PLATE 117

FIG. 13. Supravital preparation of a chromophobe pituitary adenoma showing a group of cells of about the same size with very finely granular cytoplasm.  $\times 850$ .

FIG. 14. Supravital preparation of a pituitary adenoma of mixed type. Note the size of the cells as compared with those in Fig. 13, and the granular cytoplasm stained by neutral red. One cell with two nuclei is shown.  $\times 850$ .

FIG. 15. Supravital preparation of a meningioma to show how well whorl formation is preserved.  $\times 600$ .

FIG. 16. Zenker-fixed preparation of same tumor as in Fig. 15 (eosin-methylene blue stain).  $\times 600$ .



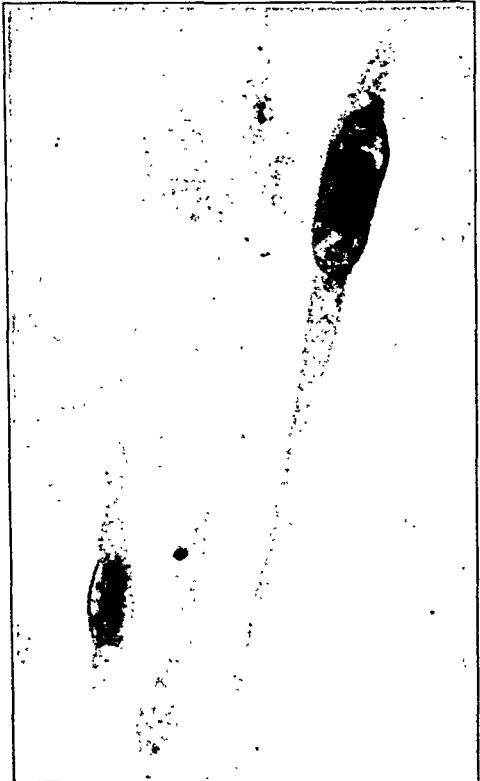
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connective tissue, and the tumor is thus said to be alveolated. In the midst of the cell masses there may be a papilliform strand of connective tissue fibers with one or two thin-walled blood vessels and the tumor cells appear to grow in parallel bundles from this fibrous tissue. Nuclear character varies greatly. The spindle cells usually have vesicular nuclei and the round cells usually densely chromatic nuclei, but the reverse may be true. Mitotic figures are not commonly abundant.

Ewing's<sup>1</sup> article on lymphoepithelioma describes tumors of the nasopharynx which histologically resemble transitional cell carcinoma, but the structure of bronchi and bronchioles gives no ground for assuming that the small cell cancers of the lung belong in the group of lymphoepithelioma.

Only within recent years have the small cell tumors of the lung been accepted as cancers, although Turnbull, who has seen an extraordinary number of lung tumors at The London Hospital, is quoted by Simpson<sup>2</sup> as having been of this opinion for many years. That they are not sarcomas has been concluded because of cellular arrangement, vascularization, connective tissue relations, gross characters resembling obvious carcinomas and distribution of metastases.

The subject is of interest in connection with the Schneeberg lung cancers. Clinically the disease was described in 1770 (see Uhlig<sup>3</sup>). In 1879 Härting and Hesse described cases which came to autopsy. Schmorl<sup>4</sup> states that these were considered to be lymphosarcoma by Wagner, by Weigert and by Anke. Arnstein<sup>5</sup> pointed out clearly that the small cell metastases of his case were epithelial. Schmorl quoted Uhlig as having demonstrated that one of the Härting-Hesse cases was a small cell carcinoma and the other was combined with an "Endothelkrebs." With this background the impression has apparently been gained that the Schneeberg tumors are commonly of the small cell type. Schmorl's examination of twenty-one Schneeberg lung cancers showed that only three of them were small cell cancers.

In some clinics the small cell tumors constitute the majority of the lung cancers, but in others their number is exceeded by the squamous epitheliomas. Of twenty-five cases of primary carcinoma of the lung observed by us at City and Lakeside Hospitals, demonstrated by complete autopsy, thirteen were squamous epitheliomas,

## SMALL CELL CARCINOMAS OF THE LUNG\*

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With the increasing number of reports upon primary carcinoma of the lung, views as to classification have changed. Classification upon the basis of gross morbid anatomy has been largely abandoned because of wide variations in form, which overlap the classes, apparently as the result of local extensions and regional metastases. Classification as to point of origin is unsatisfactory because there have been no convincing demonstrations of origin from any part of the lung other than bronchi and bronchioles.

Classification upon the basis of the histological character is not without its difficulties, but is more satisfactory than the other two modes. Squamous epitheliomas and adenocarcinomas can be readily identified. Even when the epitheliomas show no keratinization the other features are usually sufficient for a diagnosis. Often the adenocarcinomas show only a few areas of acinus formation, which may not be found in only a single section. Of the undifferentiated cancers some are made up of polygonal, cuboidal or cylindrical cells and can only be called carcinoma simplex. Others show a curious tendency to the formation of bizarre multinucleated giant cells, but it is doubtful that these justify a separate classification because such cells are found in undifferentiated and differentiated tumors of our series. Most common among the undifferentiated forms are those cancers which have been called transitional cell, oat-cell and small cell carcinomas.

Practically without exception this last group of tumors is made up of small spindle cells and small round cells, one or the other predominating in different parts of the tumor and its metastases. In the spindle cell areas a certain degree of nuclear palisading is noted but not with the same regularity of arrangement commonly seen in the neurofibroma (Schwannoma). The cells are likely to be grouped in large nests, bounded by irregular, well vascularized septa of mature

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Grossly, with the possible exception of the tumor discovered microscopically, all the small cell cancers were situated at the hilum. Two showed multiple small nodules throughout the lung. The other three were masses 6 to 12 cm. in diameter near the hilum. One case showed extensive bronchiectasis and one showed an area of necrosis (not gangrenous) 6 to 8 cm. in diameter, distal to the tumor. Generally, the tumors were of gray color, clearly invasive, firm, cut with resistance and showed a firm, pale gray or yellowish gray, moist cut surface. Vascularization was not marked and hemorrhage not noteworthy. Necrosis occurred in the larger masses as it did in carcinoma in general.

Extrathoracic metastases were proportionately more frequent in our small series of small cell cancers than in the others. Especially notable is the fact that whereas four of the nineteen other cancers showed extensive mediastinal metastasis, three of the six small cell cancers showed marked involvement of that region. Two of these were diagnosed clinically as mediastinal sarcoma. The situation in our cases, as is true of such tumors generally, was especially in the upper posterior mediastinum with anterior displacement of the trachea. This type of metastasis has been emphasized by others and Barnard<sup>7</sup> goes so far as to state that "the so-called 'oat-celled sarcoma' of the mediastinum is a medullary carcinoma of bronchi." Shennan<sup>8</sup> describes thirteen cases of small cell cancer of the lung, one of which (No. 8) is not entirely acceptable to us as of this category. All of these showed some degree of mediastinal involvement and in seven of them it was marked. Duguid and Kennedy<sup>9</sup> conclude "that oat-cell forms in a mediastinal tumor must not always be interpreted as indications of bronchial origin," and report one case originating in a thymic tumor and one originating in the mediastinal lymph nodes. Schuster,<sup>10</sup> without publishing the figures, states that "there is no relation between the type of growth and the size of the mediastinal mass." Maxwell<sup>11</sup> in a series of 239 cases of primary malignant tumor in the thorax, of which 135 were examined microscopically, found forty-seven cases of "obvious" carcinoma and sixty-four cases of oat-cell tumor. Mediastinal involvement was found in about 60 per cent of the small cell tumors as compared with about 55 per cent of the other cancers. Huguenin<sup>12</sup> speaks of "tumeurs médiastino-pulmonaires qui se présentent fréquemment sous l'aspect de tumeurs à petites cellules." It is probable

six were small cell carcinomas, four were adenocarcinoma and two were classified as carcinoma simplex.

It is at least possible that the study of primary carcinoma of the lung may be advanced by detailed examination of the different forms encountered. For this purpose the cases of small cell carcinoma have been selected. The information provided by Weller<sup>6</sup> and by Simpson provides us with data for larger comparison.

Primary cancer of the lung occurs between 20 and 80 plus years, with the greatest incidence between 50 and 60 years. In our twenty-five cases the ages were between 38 and 76 years. The small cell cancers occurred between 40 and 72 years, distributed at 40, 41, 41, 58, 64, 72 years. There is then no striking change of age incidence.

Although statistics differ, in general it may be said that the sex ratio of primary cancer of the lung is four males to one female. In our cases of small cell carcinoma four were in males and two in females.

Of our twenty-five cases nineteen were in whites and six were in negroes. Of the small cell cancers, five were in whites and one in a negro.

Duration of symptoms is based upon much human uncertainty. In Simpson's 139 cases, the average duration was seven months. Only seven lived more than two years and two more than four years. In our twenty-five cases, the duration was ten days to two and a third years. In the small cell cancer cases it was three weeks to two years.

Generally it is found that the right lung is involved slightly more often than the left. In one of our six cases of small cell carcinoma, the lesion was discovered microscopically and the side not identified. Of the others, three were on the right side and two on the left.

A study of occupation and symptoms shows nothing in which the small cell cancers differ materially from the others. Moderate anemia, leucocytosis, fever, cough, weakness, anorexia, dyspnea, hemoptysis and pain in the chest were present about equally in both groups.

The correct clinical diagnosis was made in ten of the twenty-five cases, but in the cases of small cell cancers in only one of six instances. In one of these cases it was not to be expected that the diagnosis should be made because the tumor was only about 5 mm. in diameter.

walls. Huguenin has suggested that origin from a bronchiole might easily be confused subsequently with origin from alveolar lining cells.

The problem of multicentric or unicentric origin is not solved by this observation. The bronchiolar surface involved is about 1 mm. in length. To this extent the origin appears to be somewhat diffuse as compared to the possibility of origin in a few cells. If multicentric in this instance, the number of centra must be small. Broadly speaking, this tumor can be regarded as unicentric. The examination of our entire series of lung carcinomas indicates that they originate in a bronchus or bronchiole and that multiplicity of tumors in the same lung is the result of metastasis rather than multicentric origin. This view is supported by the early invasion of lymphatics, frequent invasion of veins and occasional invasion of arteries, common to lung cancers.

The gross morbid anatomy of small cell carcinomas of the lung differs from that of other carcinomas of the lung in only two particulars. The small cell cancers are generally somewhat firmer and show a slightly greater disposition to produce large mediastinal masses than the other cancers.

Microscopically, in addition to cell type, the vascularization and desmoplastic activity of the small cell cancers is striking. The blood vessels are numerous in the pulmonary and metastatic tumors. They have thin walls, with little or no musculature and appear in delicate or heavy bands of connective tissue throughout the tumor masses. The bare capillaries or vascular slits of sarcoma are not found. With the Van Gieson stain the amount of fibrous connective tissue is seen to be in excess of what might be expected from viewing the same tissue stained with hematoxylin and eosin. Not only are there heavy bands which give the tumor an alveolated appearance, but within the tumor cell masses are many more or less delicate short or long bundles. As compared with other cancers of the lung, the amount of connective tissue in the small cell cancers appears to be greater and its distribution more diffuse.

Three of our small cell cancers have been stained with the Foot modification and one with the Maresch modification of the Bielschowsky technique. Whereas the cell masses show fibrils of connective tissue, they are devoid of reticulum. This is entirely independent of rapidity of growth as judged by clinical history or gen-

therefore that mediastinal involvement is somewhat more frequent in connection with the small cell cancers than is true of other cancers of the lung.

Discussion of the origin of primary cancers of the lung has been of particular importance as concerns the small cell tumors, for this is the type which Huguenin thought to be derived from alveolar lining. Weller, in his review, states that "proof of the origin of carcinoma of the lung from histologically unaltered alveolar epithelium is lacking." Subsequently Schuster concluded that "the existence of alveolar carcinoma is not proven." Maxwell, referring to the sixty-four small cell carcinomas he studied, states that "it has not been shown that any of the tumors in this series arose directly in the epithelial lining of the pulmonary alveoli." Huguenin, Foulon and Delarue,<sup>13</sup> as well as Huguenin, appear to admit some doubt as to Huguenin's original contention. All writers on this subject hope for what Weller calls the "fortuitous discovery of early examples in the course of routine autopsies," or the "hazard heureux de coupe" of Huguenin. Certainly the modern literature shows agreement with Weller that cell type of the tumor is not a criterion of point of origin.

Figs. 1, 2, 3 and 4 are from a small cell cancer found by Dr. Alan Moritz in the routine sections of the lung in a case of chronic diffuse bronchiectasis. In addition to masses of oval cells in the lung tissue a large space shows a lining of multiple layers of cells of the same type. That this space is not alveolar is indicated by the fact that its walls contain much smooth muscle, as shown by the round ended cylindrical nuclei. The Van Gieson technique shows many yellow-stained fibrils in the wall and the Verhoeff elastica stain several layers of elastic fibrils in irregular arrangement. The Mallory connective tissue stain shows spaces between the connective tissue fibrils. It must be a bronchiole. A slightly larger bronchiole shows squamous metaplasia without keratinization. It is probable, but not proved, that the multiple layers of oval cells represent a diffuse proliferation, but this does not exclude the possibility of extension of the cancer over the bronchiolar surface. No other focus of origin could be found in many sections, nor could a similar multiplication of layers be found in any of the clearly distinguishable alveoli. The Bielschowsky-Foot stain<sup>14</sup> (Fig. 2) shows no reticulum in the surface cells or in the deep cancer nodules. The origin of this lesion appears to be in a bronchiole and not in a bronchus with cartilaginous

Although others have observed a considerable differentiation of the cells, only one of our cases showed in one field cells which could be regarded as cuboidal or low cylindrical. Generally speaking, these small cell tumors show little or no further differentiation.

### CONCLUSIONS

1. Small cell primary tumors of the lungs or bronchi are epithelial in character, as indicated by cell arrangement, relation of connective tissues and blood vessels, and complete absence of capacity to form reticulum.

2. Small cell cancers of the lung originate in bronchi or bronchioles and are probably unicentric in origin.

3. Small cell cancers of the lung more frequently produce large mediastinal masses than do other cancers of this organ and are firmer in consistency, but in other clinical and gross pathological aspects show no distinctive characters.

eral appearance of the tumor. In no instance were reticulum fibrils found except as occasional strands connected with the reticulum of the surrounding host tissue. There is no indication whatever that the tumor cells have any capacity to form reticulum. This demonstration is in accord with that made as early as 1913 by Arnstein. Although rapidly growing sarcomas may be poor in reticulum, the lymphatic group, with which the small cell cancers have been confused, usually show a striking tendency in this direction, in sharp contrast to the small cell cancers.

The cells of the tumor appear in masses which resemble pure cultures of the cells (Huguenin). This appearance is conspicuous in hematoxylin-eosin and Bielschowsky preparations, but in Van Gieson preparations the illusion is destroyed by the presence of the connective tissue fibrils. Parallel rows of oval or spindle cells are attached to the denser connective tissue bands. These are continuous with masses of round cells or of spindle or oval cells which often occur in bundles with a certain suggestion of palisading of nuclei. There is no reason for suspecting that the round cells are simply cross-sections of spindle cells because they occur in large masses without spindle cells in association. The fact that areas of tumor necrosis are usually surrounded by round cells and that densely chromatic small nuclei, probably pyknotic, are frequent in the round cells suggests that some of these cells are the seat of regressive pathological change. Thus, they may be regressive forms of other round cells or of spindle cells. Variability of nuclear staining is a notable feature of both spindle and round cells. Nuclei may appear to be vesicular or densely chromatic. Since the nuclei of bronchiolar epithelium and the connective tissues stain normally in the sections it is probable that the tumor cell nuclei vary in chromatin content as indicated by the staining. In a specimen removed by the bronchoscope, the same irregularity of nuclear character was observed. No autopsy was permitted in this case and although the diagnosis was practically certain the case is not included in our series. Admitting it as a small cell cancer of the lung excludes the irregularity of nuclear staining as a postmortem change. The nuclei of the round cells are of the order of twice the diameter of an erythrocyte. Those of the spindle cells are of the order of one erythrocyte in width and two erythrocytes in length. Multiple nuclei are infrequent. The few mitotic figures observed are not abnormal.

## DESCRIPTION OF PLATES

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### PLATE 118

- FIG. 1. Multiplication of bronchiolar epithelium and nodule of small cell carcinoma deeper in the tissue. Hematoxylin and eosin. Green filter, Wratten B 58.  $\times 200$ .
- FIG. 2. Same material as in Fig. 1, stained by the Bielschowsky-Foot method. Note absence of reticulum in the epithelial areas. Orange filter, Wratten G 15.  $\times 200$ .
- FIG. 3. Same material as Fig. 1, stained by the Verhoeff elastica method. Note the irregular distribution of fibrils, different from that of an artery. Red filter, Wratten A 25.  $\times 200$ .
- FIG. 4. Same material as Fig. 1, stained by the Mallory connective tissue stain. Note the wide spaces between the fibrils in the subepithelial tissue. The spaces are occupied by smooth muscle as demonstrated by the Van Gieson method. Green filter.  $\times 200$ .

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PLATE 119

- FIG. 5. Spindle cells and round cells in small cell carcinoma in lung. Note shrunken connective tissue trabeculae. Hematoxylin and eosin. Green filter.  $\times 200$ .
- FIG. 6. Same tumor as in Fig. 5, stained by Bielschowsky-Foot method. Note absence of reticulum in the tumor nodules. Orange filter.  $\times 200$ .
- FIG. 7. Hepatic metastasis of small cell tumor of lung. Hematoxylin and eosin. Green filter.  $\times 200$ .
- FIG. 8. Area near that shown in Fig. 7 stained by Bielschowsky-Foot method. Note absence of reticulum in tumor nodules. Orange filter.  $\times 200$ .



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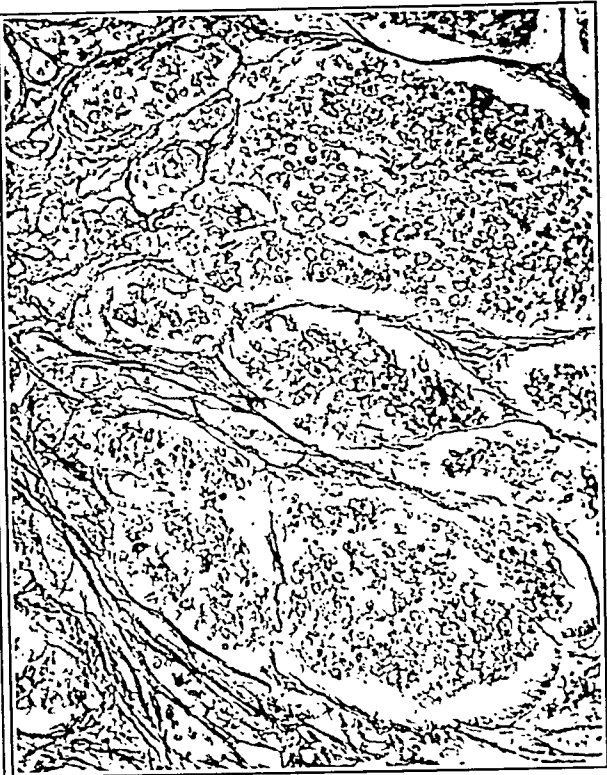


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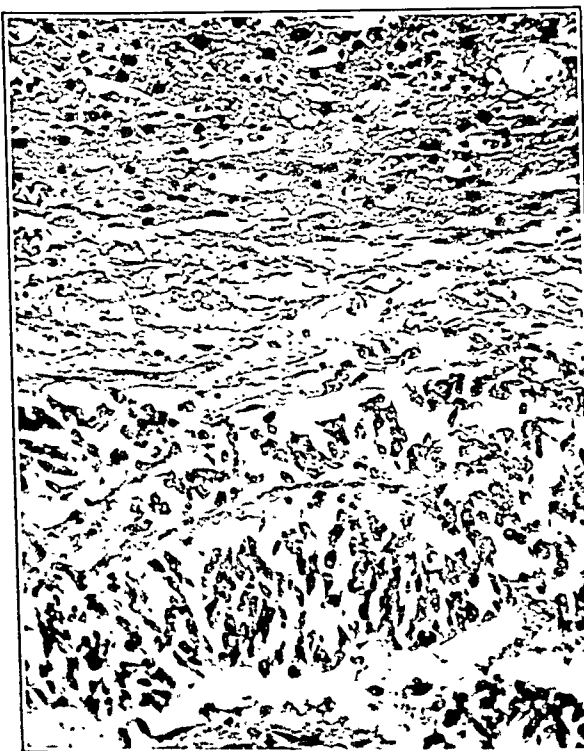




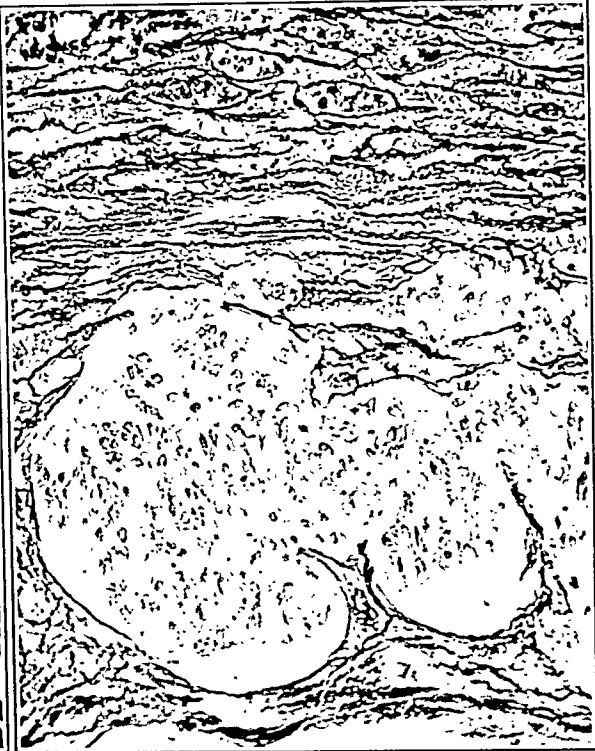
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Pathologists lay entirely too much stress on the channels of transportation in the formation of metastases generally, and particularly in the predilection of certain types of carcinoma to metastasize in the bone.

Von Recklinghausen,<sup>5</sup> who was the first to investigate the subject thoroughly, maintained that the formation of metastases in the bones is due to the fact that the veins and capillaries of the bone marrow have thin walls, and are not collapsible. These morphological peculiarities favor the accumulation of carcinoma cells within these blood vessels. The majority of pathologists side with von Recklinghausen in the opinion that the mechanical peculiarities are the main, if not the only factors in the causation of skeletal metastases. None the less this hypothesis is inadequate to explain all the phenomena connected with the formation of skeletal metastases. Kaufmann,<sup>6</sup> in a detailed account of a statistical investigation of the frequency of metastases in the bone, found skeletal metastases in carcinoma of the uterus in 3.03 per cent and in carcinoma of the prostate in 66 per cent. The difference between 3.03 per cent of skeletal metastases in carcinoma of the uterus and 66 per cent in carcinoma of the prostate is very striking, and cannot be explained by mechanical differences. The topographical relationships between the uterus and the skeleton on one hand, and the prostate and the skeleton on the other, are practically identical. So is the vascularization of the two organs, and it is as easy for the cancer cells from the uterus as those from the prostate to find lodgment in the bone marrow.

The opinion of Lubarsch<sup>7</sup> that the difference in the relative sizes of the cancer cells and of the minute blood vessels of an organ influence the formation of metastases, is also hardly tenable. The bone marrow contains blood vessels of a sufficiently large caliber to admit the lodgment of a cancer cell of any size.

The only plausible explanation of this difference between tumors of certain organs seems to be that while both the cells of the uterine carcinoma and the carcinoma of the prostate find lodgment in the marrow, the latter cells find there a better soil for development than do cells of the uterine carcinoma. A very striking instance indicating the existence of a peculiar affinity between the cancer cells and bone tissue was presented by Schmorl.<sup>8</sup> He found in a series of cases of carcinoma, metastases not only in the bones of the skeleton, but in bone tissue abnormally developed in the cartilage of the

## SKELETAL METASTASES IN CARCINOMA OF THE THYROID \*

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Following carcinoma of the prostate and of the breast, carcinoma of the thyroid most frequently metastasizes in the skeleton. Berard and Dunet<sup>1</sup> in their recent analysis on the subject found the frequency of skeletal metastases in carcinoma of the thyroid to vary between 18, 30 and even 70 per cent of all cases. On further analysis of 110 cases of skeletal metastases in carcinoma of the thyroid, they found that the most frequent sites of the metastases were the skull and the vertebrae.

There are several characteristics in the secondary metastatic tumors of carcinoma of the thyroid which make them the most favorable material for the study of the general problem of metastasis formation.

Ewald<sup>2</sup> showed the presence of iodine in metastatic adenocarcinoma of the thyroid while the original tumor was free from iodine.

A simple colloid goiter, presenting a structure that usually remains harmless for years, may give rise to metastatic tumors. These secondary tumors may acquire the characteristics of malignancy though the primary tumor remains benign.

Riedel<sup>3</sup> removed a tumor of the inferior maxilla composed of normal thyroid tissue. The tumor, however, recurred locally after ten years.

In order to elucidate these apparent peculiarities in the formation of metastasis generally, and particularly of skeletal metastasis in carcinoma of the thyroid, a brief survey must be made of the mechanism of formation and pathogenesis of metastasis in carcinoma generally.

The writer<sup>4</sup> studied the problem experimentally and clinically. He also analyzed microscopically a large number of cases of skeletal metastasis. The conclusions reached from this combined study do not always coincide with the opinions of the investigators of purely anatomical material.

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noted frequently around large tumor masses but not around minute metastatic nodules. In the latter instance, as stated above, no morphological abnormality could be found in the bone marrow.

As the tumor nodule increases in size it approaches and invades the compact osseous tissue or the compact osseous partitions of the cancellated bone. Then, there begin to appear the characteristic changes in the bone tissue. It is generally accepted that there are two classes of skeletal metastases of carcinoma: *osteoplastic*, in which extensive new bone formation takes place around the metastatic tumor; and *osteoporotic*, in which the changes in the normal tissue surrounding the metastasis consist in extensive destruction of the compact bone. These two distinct conditions can be easily differentiated on the gross inspection of skeletal metastases. However, the microscopic study of the cases analyzed by the writer showed that both conditions were generally present side by side. The differences in the gross appearance are due to the fact that in one case osteosclerosis or new bone formation predominates, while in another osteoporosis or the destruction of the old bone is mostly in evidence.

The mechanism of the bone destruction in metastases of carcinoma differs from the one observed in inflammatory osteoporosis. Von Recklinghausen first made the observation that the large polynuclear osteoclasts, which destroy the bone in osteoporosis, are very seldom found in the lacunae of the bone surrounding a growing metastasis of carcinoma. This fact was confirmed by most of the subsequent investigators. In view of the absence of the large osteoclasts, von Recklinghausen presumed that there takes place in this bone a softening by the removal of its inorganic salts and a subsequent absorption without the aid of any cells, a condition similar to the one found in osteomalacia. Apolant,<sup>9</sup> Erbslöh<sup>10</sup> and Askanazy<sup>11</sup> are also of the opinion that osteoporosis in skeletal carcinoma may take place without the aid of special cells. On the other hand, Goetsch<sup>12</sup> believes that the cancer cells act as osteoclasts and destroy the compact bone, and Axhausen maintains that the small elongated mononuclear connective tissue cells frequently found close to the walls of the lacunae are special osteoclasts derived from the cancer stroma. In the specimens studied by the writer both cancer cells and the small connective tissue stroma cells were found in immediate apposition to the walls of the lacunae. The cancer cells were so frequently the only cellular elements within the lacunae of the bone

larynx and trachea, and in one case a metastasis developed in a bone plaque on the wall of the aorta. In the latter case no metastases were formed in any organ with the exception of the bones of the skeleton and of the bone tissue in the wall of the aorta. It is inconceivable that cancer cells should have been able to reach the small bits of bone tissue of the aortic plaque more easily than the parenchymatous organs.

Thus the probabilities are strongly in favor of the conception that tumor emboli are transported everywhere with the same ease and find lodgment in any part or organ of the body. On the other hand, the further growth of the small transported group of cancer cells and their development into a large metastatic tumor depends upon a correct affinity between the cancer cells on the one hand, and the organ cells, the soil as it were, on the other.

The writer has shown in a series of investigations that the development and growth of inoculable malignant tumors of the lower animals depend upon a local interaction between tumor cells and the somatic cells of an organism. In certain instances the parenchymatous cells of a normal organ may inhibit the growth of the tumor, and this inhibition ceases when the parenchymatous cells become degenerated through a certain injury to the organ. On the other hand, the writer has shown that when the cells of a normal organ offer no adequate resistance to the growth of the tumor, then the proliferating tumor cells injure, mechanically or chemically, the former cells. This injury evidently precedes the death of the organ cells and their replacement by tumor cells.

Bone tissue, in view of its characteristic morphological differences from all other tissues, presents a very favorable object for the minute study of the changes taking place in the soil under the influence of tumor growth.

A microscopic study of skeletal metastases of carcinoma which the writer undertook some time ago brought out several points of importance.

The metastasis begins its development within the marrow, and when the group of cancer cells is small, the surrounding bone marrow appears quite normal. Von Recklinghausen claims that the development of the metastasis is preceded by a hyperemia and hemostasis, due to obstruction of the capillaries by the tumor emboli. In the specimens studied by the writer, hemorrhages and hyperemia were



tween the normal organ tissue and the cancer cells during the beginning of the development of a metastatic tumor from a transported cancer embolus. Upon the result of this interaction depends the success of metastasis formation.

This conception of the pathogenesis of formation of metastases may well serve to clarify the meaning of the peculiarities encountered in the metastases in carcinoma of the thyroid. It is impossible to conceive that a normal cell of the thyroid or a cell of a benign goiter when transplanted into a bone may form a malignant tumor. On the other hand, it is often difficult to differentiate morphologically between a benign and a malignant tumor of the thyroid. It was stated above that the failure or success of the development of a metastatic tumor from a transported embolus depends upon the interaction between the normal organ tissue and the cancer cells.

We may conceive that a small group of cells of a normal thyroid or of a benign goiter may become malignant, but the thyroid or goiter offers an unfavorable soil and suppresses the further development of a malignant tumor for a longer or shorter period of time. The same small group of malignant cells when severed from the primary locus and transported to bone may find there a more favorable soil and develop into a large, actively growing metastatic carcinoma. Moreover these cancer cells may not only increase its power of proliferation but may also increase its functional capacity. Thus Ewald noticed that iodine is produced in the metastatic tumor while none was produced in the primary tumor.

Cases in which the primary tumor in the thyroid is small and not malignant clinically, while the metastatic tumor is large and very highly malignant, are encountered frequently in carcinoma of the thyroid.

This is not only of theoretical, but also of great practical importance. The whole symptom complex is caused by the metastatic tumor, therefore the primary condition is frequently overlooked and both the diagnosis and therapy may be wrong.

The cases of skeletal metastases in carcinoma of the thyroid reported here all belong to this type.

#### CASE REPORTS

CASE 1. *Clinical History:* M. B., white, male, aged 31 years, was seen at the clinic in 1924, suffering from epithelioma of the ear. He disappeared and presented himself again three years later.

that there cannot be any doubt but that cancer cells do act as direct osteoclasts. The impression gained by the writer is that the small stroma cells only subsequently invade between the bone and the cancer cells. But even if the possibility be admitted that occasionally the stroma cells may act as osteoclasts, it seems quite apparent that the tumor itself, by the aid of its formed elements, first destroys the bone, and then grows by occupying the space so formed.

The mechanism of osteoporosis in skeletal metastases is similar to the mechanism of the destruction of any normal tissue surrounding a growing malignant tumor; the osteosclerosis or the extensive formation of new bone tissue is similar to stroma formation within and around a metastatic tumor.

Von Recklinghausen claims that this extensive new bone formation is due to the hyperemia described by him and mentioned above. Askanazy thinks that the metastasis at first produces an osteoporosis which is followed by a bone necrosis. The necrotic bone acting as a foreign body causes a new bone formation. Wolff,<sup>13</sup> and Kaufmann are of the opinion that cancer cells may act directly as osteoblasts and form new bone tissue.

The mechanism of the new bone formation as observed by the writer is as follows: At first, collagen fibrils are formed in abundance from the old bone. These fibrils gradually unite in thick bundles and subsequently form new bone tissue. The latter is clearly formed from the constituent parts of the old bone tissue. On the other hand, as stated above, the writer did not observe any hyperemia, inflammation, or any other abnormality of the bone marrow at the beginning of the development of the metastases. Furthermore, necrosis of the bone was not found in any of the specimens examined. It must be concluded that some unknown chemical irritant emanating from the cancer cells acts on the old bone tissue and stimulates its proliferation.

Thus two processes always take place side by side in skeletal metastases. On the one hand, the tumor destroys the normal bone tissue, on the other hand the remaining osseous tissue proliferates and creates new bone. The latter is quite probably an attempt at self defense. The new bone tissue may compress and destroy the cancer cells.

The minute microscopic study of skeletal metastases gives the clearest evidence of the interaction which always takes place be-

ary to metastatic carcinoma of vertebrae (paraplegia dolorosa Charcot).

**CASE 2. Clinical History:** A. K., white, male, aged 73 years. In September 1928, the patient developed an acute swelling in and around the left sterno-clavicular junction. A few days later a similar swelling developed on the other side and both swellings disappeared in a short time without treatment. A few days later, another swelling appeared in what seemed to be the region of the right lobe of the thyroid. Later, a similar condition appeared on the other side. All the swellings disappeared in a short time. In May, 1929, the patient began to suffer from constant pain in front and back of chest accompanied by coughing and bloody expectoration and also by a high temperature. This acute condition subsided in about ten days. During this period, swellings appeared in the cervical region. About four weeks ago, the patient suddenly felt pressure pain in the back region immediately below the angle of the scapula. This pain continued up to the present. Occasionally, this condition came in spasms, with comparative comfort in between. At present, the greatest pain he has had is in a recumbent position. Patient did not suffer from constipation, had normal bladder function and a fair appetite.

**Physical Examination:** The patient was emaciated, showing evidence of cachexia and had the appearance of severe muscular tension. In the right supraclavicular region a few lymph nodes were palpated. There was a lymph node noted in the region of the mastoid which was very firm and measured 2.5 cm. in diameter. Another was palpated above the sternocleidomastoid region, which measured 4 cm. in diameter, was hard and nodular, and firmly attached to the underlying structure. There were other lymph nodes noted in both axilla, larger in the left. There were some small inguinal lymph nodes, more on the right side. No other abnormalities were noted.

Results of an X-ray examination of chest and skeleton showed the following conditions:

There was an osteoporosis and decalcification observed in the ribs, spine, pelvis and upper ends of the femora. In addition, there appeared an irregular, destructive process in the superior surface of the second lumbar vertebra with a definite upward concavity.

In the right lung there was noted an irregular infiltrative and fibrotic involvement, especially in the upper lobe, with some bronchiectatic and interstitial pneumonic changes appearing at the right base. Interlobar plastic pleurisy appeared at the level of the fourth rib anteriorly. In the left side, the lung showed a similar infiltrative change in the upper lobe. In addition, at the level of the third rib, there appeared a small circular shadow of increased density measuring about 1 cm. in diameter. A similar nodule about 2.5 cm. in diameter was noted at the level of the sixth rib anteriorly.

**Diagnosis:** Polyadenitis noted in this case with a history of swellings in the neck appearing and disappearing without treatment is a symptom complex which would indicate that we were dealing with lymphoma malignum (Hodgkin's disease). The two discrete metastatic nodules noted in the X-ray examination may also have been lymphomatous nodules, but the multiple conditions in the skeleton

At this time the original condition seemed to be improving. About nine months ago an insidious painless swelling arose over the region of the right clavicle. This condition grew slowly but gave the patient no discomfort. Three months later, the patient began to complain of cramp-like pain in the spine. This pain occurred in the daytime, usually at the onset, and was brought on by change of position. In addition to this, the patient complained of pain in the upper spine between the shoulder blades. During this time there was also noted some loss of weight.

Two months later the patient noticed a lump in the occipital region. Other lumps arose in the region of the scalp.

About five weeks ago the patient noticed shooting pains in the legs, originating in the spine. Gradually, power in the lower extremities began to disappear, first on the left side and later on the right.

*Physical Examination:* General appearance was that of a young adult male about 30 years of age. He presented an anxious expression and appeared to be suffering from recurring pains. He appeared anemic and cachectic.

The right ear was distorted and much smaller than the left. The skin behind the ear was depressed, soft and smooth. The external auditory meatus was occluded by a flat ulcerated neoplastic area 12 by 8 mm. in extent. This area bled readily to the touch and was pinkish in color and granulation-like in appearance.

The chest showed a distinct flattening anteriorly in the region of the nipples. Clavicular regions showed an asymmetry. The right clavicle was much more prominent. On palpation the clavicle was replaced by a soft and elastic, smooth mass about 6 by 3.5 cm. in extent. On palpation the left clavicle also presented many protuberances and depressions. On palpation of the chest, several protuberances were felt.

Over the region of the anterior mediastinum, percussion revealed an area of dullness reaching about an inch to right and left of the sternal margins.

Over the regions of the third, fourth and fifth dorsal vertebrae there was a protrusion and widening of the spine. A similar deformity was felt over the lower lumbar spine and sacrum. The right humerus contained many nodules over its surface as did the left, but to a lesser degree. Both femora presented bony irregularities, particularly in the upper portions.

The patient died on April 26, 1927 and no autopsy was obtained.

*Diagnosis:* The clinical analysis of the case shows that the polypoid-like structure in the external ear which appeared like exuberant neoplastic tissue was apparently not the epidermoid cancer of skin with which the patient suffered three years ago, but was metastatic in nature. The dull area of the anterior chest began about 1 inch to the right and ended 1 inch to the left of the sternum. In the upper margin it began at the tip of the sternum and went down for 2 inches. The position of the dullness showed definitely that the tumor mass was situated neither in the mediastinum nor in the lungs, but in the intrathoracic part of the neck and consequently represented a carcinoma of an intrathoracic thyroid, with multiple osteoporotic metastases in the skeleton. The neurological condition was second-

## SUMMARY

1. Three cases of metastases in the skeleton secondary to carcinoma of the thyroid are reported.

2. In all three cases the clinical symptom complex as well as the evident pathology was caused by tumors in the bone. While the primary tumor in the thyroid was insignificant, both pathologically as well as clinically, compared with the condition in the skeleton, as a result in all three cases, the primary condition was overlooked.

3. Whenever a diagnosis of malignant tumor in the skeleton, single or multiple, is made, a search must be undertaken for a primary malignant tumor elsewhere before the diagnosis of a primary malignant tumor in the skeleton can be made.

4. In such a search for a primary malignant tumor, in a female, next to the breast, the thyroid must be thought of, and in a male, next to the prostate, the thyroid must be considered as the most probable seat of a primary tumor. Such a diagnostic analysis is of importance not only from the theoretical but also from the practical clinical standpoint.

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did not coincide with Hodgkin's disease and a biopsy of the nodule which was situated in the right lobe of the thyroid microscopically showed it to be a papillary adenocarcinoma of the thyroid.

In view of the pathological findings, the conclusion was reached that the symptom complex, particularly the severe pain in the back which was the major complaint of the patient, was due to the carcinoma of the second lumbar vertebra, secondary to papillary adenocarcinoma of the thyroid.

*CASE 3. Clinical History:* White female, aged 38 years. The patient was admitted to the clinic in February, 1927, complaining of pain in the lower part of the back, which radiated mainly to the left thigh. The pain had continued for four months. Patient also complained of irregular menstruation which had continued for the last three years. She was referred to the clinic after a diagnosis of carcinoma of the cervix was made outside the hospital.

*Physical Examination:* Patient appeared to be well nourished. No adenopathy noted. Thyroid appeared enlarged but seemingly encapsulated and freely movable throughout. Examination of the pelvic organs showed no malignancy in the genito-urinary organs.

X-ray analysis showed an area of irregular rarefaction on the left side of the sacrum involving the first and second sacral segments. The neurological examination seemed to point to a tumor in the lower spine. Subsequent examination of pelvic bones showed that rarefaction was gradually increasing in extent.

*Diagnosis:* The problem to be solved was whether the rarefaction shown by X-rays was due to a primary sarcoma of the sacrum or metastatic carcinoma with primary seat located elsewhere. It seemed rather unusual for sarcoma of the sacrum to continue for so many months, or for a tumor mass to grow out of the shell of the sacrum and produce a palpable mass.

Examination showed no malignancy anywhere. The only pathological condition encountered was a small, freely movable goiter. Since this goiter may have been the seat of malignancy and if so would cause an additional severe symptom complex if allowed to develop further, a partial thyroidectomy was done and microscopic examination showed it to be an adenocarcinoma of the thyroid.

Subsequently, in an attempt to arrest the rapid growth of the sacral tumor and also in order to control severe pain, a partial excision with insertion of radium was done. Microscopic analysis of the tissue removed showed the identical picture of the thyroid carcinoma. The condition grew worse, notwithstanding all therapy, and the patient died.

*Autopsy Report:* The body is that of a well developed and well nourished negro child, 51 cm. in length and weighing 2500 gm. The skin and mucous membranes are clear, moist and elastic and show no signs of desquamation, eruption or fissures. There is no glandular adenopathy or indication of birth injury. The eyes, ears, nose and throat are normal. The chest shows no deformation. The abdomen is not distended. From the recently ligated umbilical cord there is no sign of hemorrhage. The external genitalia, anus and extremities are normal. The peritoneum and pleura are clean and glistening; neither peritoneal nor pleural cavities contain exudate, transudate or adhesions. The thymus and thyroid are normal. The lungs are of normal size, color and consistency. The spleen is slightly congested. The intestinal tract, pancreas, adrenals, bladder and lymphatic system are normal. Grossly, the epiphyses of the long bones show no syphilitic osteochondritis. In the pericardial cavity are approximately 5 cc. of slightly blood-tinged fluid but no indications of acute or chronic inflammation. The epicardium and coronaries appear normal, and on removal the heart weighs 25 gm. The pulmonary artery contains a small postmortem clot. The heart walls are firm, of good color and consistency and in the heart cavities are small amounts of coagulated blood. The foramen ovale is closed and the valves, chordae tendineae and endocardium appear normal. On section of the left ventricular wall, an oval, grayish white nodule measuring 1.25 by 0.5 by 0.5 cm. is seen, which extends from just below the epicardium to the endocardial lining and is situated adjacent to the interventricular septum on the anterior aspect of the heart. Two similar nodules 0.5 cm. in diameter are noted adjacent to the interventricular septum midway between the apex and the auricular ventricular partition, one in the anterior wall of the right ventricle and the other in its posterior wall. These nodules are firm, swollen, well demarcated, protrude slightly and reveal a small area of softening at their centers.

*Histopathology:* Sections of lesions of the heart fixed in 10 per cent formalin and stained by Levaditi's method reveal countless spirochetes peppered throughout. In the accompanying figure some of the treponema are seen in focus while others appear as black specks. They are especially abundant in the intercellular spaces, around the blood vessels, and in places appear incorporated in the heart muscle fibers.

## MULTIPLE GUMMAS OF THE HEART IN THE NEW BORN \*

JOHN W. WILLIAMS, M.D.

(From the Department of Pathology, Tulane University School of Medicine,  
New Orleans, La.)

A search of the literature reveals a limited number of cases of syphilis of the heart in the new born that are characterized by the lesion known as gumma. In the majority of the cases reported there are lesions of syphilis other than in the heart. Virchow,<sup>1</sup> in 1858 is probably the first to study lesions similar to the one herein described, which he terms foci of syphilitic interstitial myocarditis. Le Count<sup>2</sup> in 1898, in citing a case of congenital syphilis of the heart in a patient who died shortly after birth, says: "The title of gummata is simply a matter of preference; multiple foci of interstitial myocarditis with leucocytic invasion, areas of degeneration of the heart muscle and the occurrence of multinuclear cells resulted in areas which to the naked eye were limited in extent, whitish, and appeared softened, in other words, gummatous." Hektoen,<sup>3</sup> Adler,<sup>4</sup> Parkinson,<sup>5</sup> and Blacklock and M'Cluskie<sup>6</sup> describe similar cases. Warthin<sup>7, 8, 9, 10</sup> in his publications discusses syphilis of the heart in detail and emphasizes the importance of careful examination in congenital syphilis, citing cases in which the syphilitic involvement was limited to the heart and occurred in no other parts of the body. In a later paper he states: "This form of syphilitic disease of the heart is an important cause of asphyxia neonatorum and unexplained sudden death in early life." The syphilitic heart lesion which I am reporting is similar to the one Warthin<sup>8</sup> terms "myxogumma" and is interesting because there is no other evidence of syphilis in the body.

### CASE REPORT

*Clinical History:* The patient was a full term, negro female infant, aged 3 hours. Delivery was by breach; however, it was not difficult nor was there injury to mother or child. Since the condition of the child at birth was poor, incubation was resorted to and oxygen inhalations given. The mother's Wasserman was strongly positive, but she gave no history of previous illness. She had been pregnant once before and was delivered of a healthy child.

\* Received for publication May 16, 1930.



In the case of the heart, we are dealing with an organ whose metabolic activities are great and whose cells are highly specialized. In the event that a small infected thrombus blocks a terminal branch of one of its coronaries and a severe inflammatory reaction results, the veins through drainage of the blocked-off area decrease the tissue tension of the heart muscle while the generation of toxic material with its resultant inflammation causes an increased tension. This augments the vulnerability of muscle cells to the effect of toxins and to the action of proteolytic ferments elaborated from the dead cells of the acute reaction. The result is a lesion similar to the one seen in this case. The bits of worm-eaten, dimly striated muscle fibers with nuclei intact and the accompanying cellular reaction are proof of its acuteness and of the enzymatic nature of the destruction wrought. It is therefore more correct to regard this lesion as one of localized syphilitic cellulitis and fulminative syphilitic myositis. This terminology is preferable to that of "gumma" since cellulitis and myositis accurately designate the character of the lesion. Gumma, as we understand it, is the broken-down and encapsulated syphilitic lesion. While it is possible to have true gummas of the heart muscle, none of the so-called cases reported in the new born and herein cited conforms microscopically to our conception of the gumma.

#### SUMMARY

1. A case of gumma of the heart in a negro infant that died a few hours after birth is reported.
2. The term gumma as descriptive of the lesion is questioned since the microscopic picture is at variance with that of a gumma.
3. The terms localized syphilitic cellulitis and fulminative syphilitic myositis are suggested in its stead as descriptive of this lesion since it is characterized by muscle destruction and infiltration with lymphocytes, polymorphonuclear leucocytes, monocytes and plasma cells.

Shrunken muscle fibers, prominently striated, are seen in sections stained with hematoxylin-eosin after Zenker fixation. In areas, varying amounts of a mucoïd and vacuolated substance separate the muscle fibers. This substance has the appearance of débris and contains bits of striated muscle fiber, lymphocytes, plasma cells, monocytes and polymorphonuclear leucocytes and impresses one with the fact that muscle tissue which might once have been present had been dissolved and its place partially filled with the cells mentioned above. Other areas of muscle fibers show considerable fatty degeneration. Around the small arterioles is seen proliferation of the adventitia and infiltration with lymphocytes, plasma cells and neutrophils. There is proliferation of the subepicardial tissues and infiltration with lymphocytes, plasma cells, monocytes and polymorphonuclears. Considerable congestion is noted in all areas examined and the capillaries appear extremely numerous. No endarteritis is found and no multinuclear cells are apparent.

Microscopic examination of the liver, spleen, kidneys, suprarenals, lymph nodes and lungs shows no evidence of syphilitic lesions.

### DISCUSSION

It is interesting to note that only a limited number of congenital syphilitic heart cases have been reported which have the gross appearance of gumma but whose lesions, studied microscopically, fail to substantiate this diagnosis. Probably not more than twenty cases of this type have appeared in the literature. The similarity of location of most of the lesions is an outstanding factor. They occur adjacent to the interventricular septum in regions supplied by small branches of the posterior descending branch of the right coronary, and of the anterior descending branch of the left coronary artery. This leads one to believe that involvement of such a small branch is responsible for the location of the lesion in the musculature and that the mechanics of the coronaries can explain the branches involved.

The lack of a definite category in which to place this lesion is also of interest. Warthin prefers to call it "myxogumma." The term is somewhat vague since so little is known of the chemical nature of mucins and muscle proteins and since, in any extensive lesion of the heart in which blood, thrombi and fibroblastic proliferations occur, the mucin reaction is apt to be obtained.

## DESCRIPTION OF PLATE

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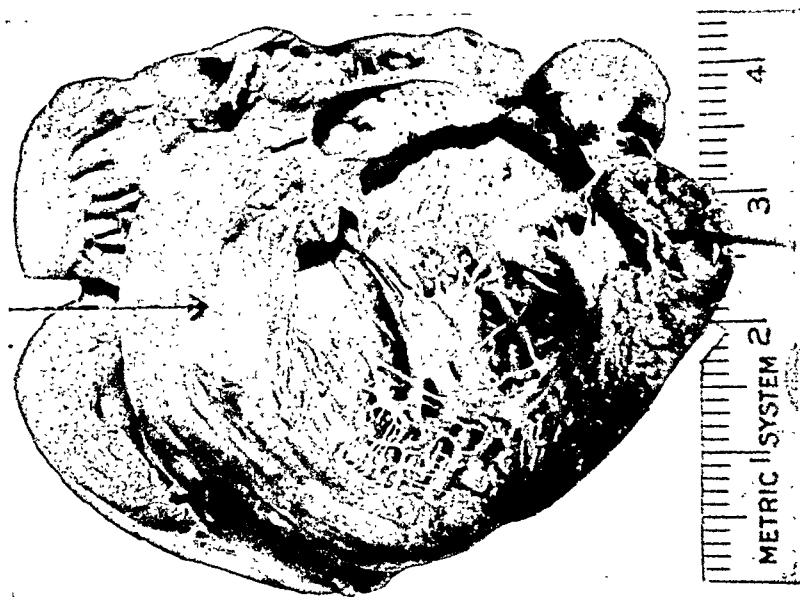
### PLATE 120

- FIG. 1. Enlarged photograph of heart. Note circumscribed syphilitic nodule in anterior wall of left ventricle adjacent to septum with area of softening in center appearing slightly darker.
- FIG. 2. Section of heart muscle stained by the Levaditi method. Note innumerable spirochetes diffusely scattered throughout the lesion.
- FIG. 3. Section of heart, Zenker fixation, hematoxylin-eosin stain. Note translucent, vacuolated areas of fatty degeneration of muscle fibers, separation of individual fibers and lymphocytic infiltration around blood vessels and between muscle fibers.
- FIG. 4. Section of heart, Zenker fixation, hematoxylin-eosin stain. Note shrunken, well striated muscle fibers separated by infiltration of lymphocytes, plasma cells, monocytes and polymorphonuclear leucocytes.
- FIG. 5. Section of heart, Zenker fixation, hematoxylin-eosin stain. Note large area with lymphocytes, monocytes, plasma cells, neutrophiles and degenerated muscle fibers with nuclei.

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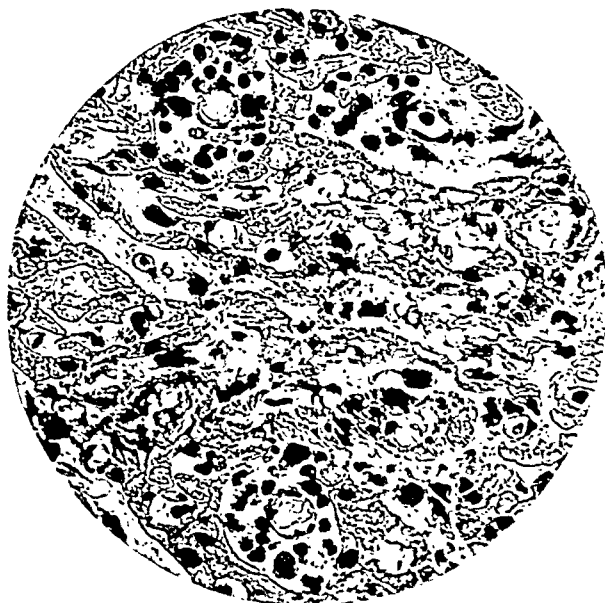




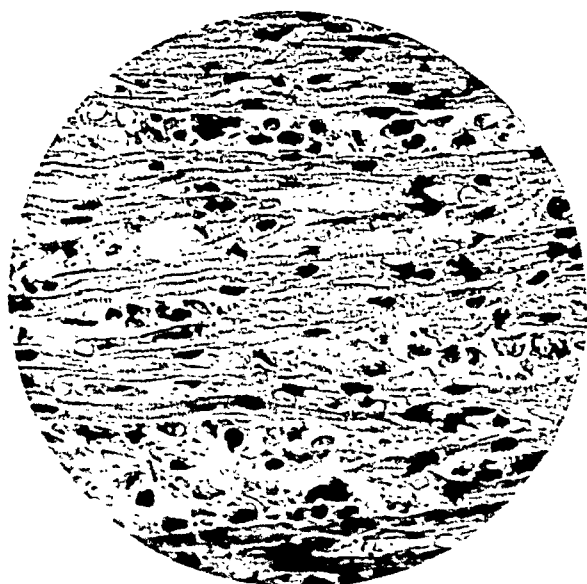
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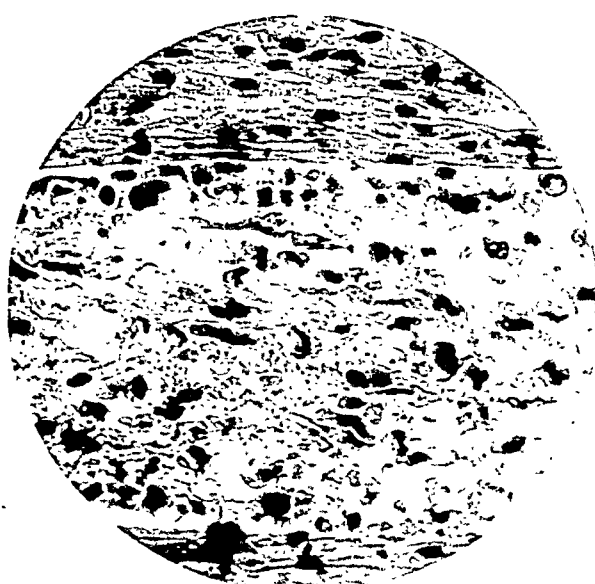
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SCIENTIFIC PROCEEDINGS OF THE  
THIRTIETH ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS AND  
BACTERIOLOGISTS

NEW YORK CITY  
April 17 and 18, 1930



Voted to record with regret the deaths of

Dr. Henry Albert

Dr. Claribel Cone

Dr. Lydia M. DeWitt

Dr. S. H. Gilliland

Dr. Paul A. Lewis

Dr. H. T. Marshall

Dr. Benjamin Roman

The Thirty-first Annual Meeting of the Association will be held at Western Reserve University, Cleveland, Ohio, April 2 and 3, 1931.

The special topic will be "Disease of the Liver — Exclusive of Tumors." Special papers will be presented by Drs. F. B. Mallory and G. H. Whipple.

The Gold Headed Cane of the Association was conferred upon Doctor Theobald Smith for distinguished service to pathology and bacteriology.

ABSTRACT OF BUSINESS SESSION  
AMERICAN ASSOCIATION OF PATHOLOGISTS AND  
BACTERIOLOGISTS

Voted to elect the following officers:

<i>President</i>	GEORGE R. CALLENDER
<i>Vice-President</i>	WARD J. MACNEAL
<i>Treasurer</i>	FRANK B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	OSWALD T. AVERY
<i>Assistant Secretary</i>	ROBERT A. MOORE

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ABSTRACT OF MEETING OF THE COUNCIL  
AMERICAN ASSOCIATION OF PATHOLOGISTS AND  
BACTERIOLOGISTS

Voted to elect the following new members: Nicholas M. Alter, Louis Berger, Zera E. Bolin, Charles F. Branch, W. W. Brandes, Carl J. Bucher, Rafael Dominguez, Norbert Enzer, Hamilton R. Fishback, Jacob Furth, Samuel H. Gray, Robert G. Green, Francis D. Gunn, Ernest M. Hall, Kiyoshi Hosoi, N. Paul Hudson, Wilhelm C. Hueper, Benjamin S. Kline, G. Kenneth Mallory, Jessie Marmorston-Gottesman, Leone McGregor, Valy Menkin, Aura J. Miller, Nickolas W. Popoff, William A. Starin, Bindo deVecchi, Cecil Watson, Charles E. Woodruff, and Arthur W. Wright.

Voted to accept the resignations of Drs. Katharine R. Collins, W. G. MacCallum, Joseph H. Pratt, James S. Simmons, and Paul G. Wooley.

sensitize another normal guinea pig. Further, if you could take large amounts of human serum and inject it into normal guinea pigs, you might be able to accomplish this transfer with greater regularity, but you cannot use much more than one or two cubic centimeters. Dr. Grove's remarkable instance of the passive transfer from a human being to a monkey is striking in that she used a very large amount of serum, and the fact that this animal had terrific dyspnea for fifty minutes. This was relieved by adrenalin. This animal was given the same injection three weeks later, and was in a refractory state which Dr. Grove speaks of in a footnote. In the conclusions to the paper she denies the importance of that striking experiment. I believe that there are many factors which we have to clarify, and I certainly do think that instead of criticizing the situation, we ought to continue attempting this transfer, and bring forth further evidence.

SKIN REACTIONS TO THE SOLUBLE TOXIC SUBSTANCE OF THE COLON BACILLUS.  
Bernhard Steinberg, Toledo, O.

*Abstract.* Skin reactions to the soluble toxic substance and to the washed, killed bacterial bodies of a twenty-four-hour culture of a colon bacillus were made. Reactions read at the end of twenty-four hours appeared in a large number of normal adults and children who were tested. In people with colon bacillus infections, the reactions to the soluble toxic substance and to the vaccine were either absent or greatly diminished in size and degree. It is assumed that the skin reaction is of an antigen-antibody mechanism. Only occasionally a normal person had agglutinins against the colon bacillus. Immunization with the soluble toxic substance of the colon bacillus reduces the size and degree of the skin reaction against it. The soluble toxic substance has apparently weak antigenic properties.

*Discussion*

(Dr. E. E. Ecker, Cleveland.) I was very much interested in the work of Dr. Steinberg and congratulate him. There is only one question I should like to ask him, and that is whether he saw any infiltration in these areas, because we found that by repeated intradermal injection with these products into animals we were never able completely to reduce the size of the inflammatory process. There was a reduction in size, but the areas became indurated. It is what Opie calls a local anaphylaxis, and I wonder if Dr. Steinberg has seen it in his human experiments.

(Dr. Steinberg, closing.) I had a personal conversation with Dr. Ecker prior to the meeting, and told him that in some instances we did find slight induration of the skin of these people who presented a negative reaction. I understand that Dr. Ecker is under the impression that he is dealing with an allergic state in some of his animals. I do not know whether I can or cannot subscribe to that opinion yet, in the present state of my work.

THE TYPE DISTRIBUTION OF MENINGOCOCCI IN THE UNITED STATES DURING  
1928-1929. Sara E. Branham, Washington, D. C.

(*Abstract not received.*)

IMMUNOLOGIC STUDIES IN BLASTOMYCOSIS. Anna Dean Dulaney, Memphis,  
Tenn.

(*Abstract not received.*)

## AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

FURTHER STUDIES ON THE PRECIPITATION TEST FOR SYPHILIS. Emil Weiss,  
Chicago, Ill.

*(Abstract not received.)*

THE IDENTITY OF ANIMAL ANAPHYLAXIS AND HUMAN ALLERGY (PROTEIN  
HYPERSENSITIVENESS). Bret Ratner and (by invitation) Helen L. Gruehl,  
New York City.

*Abstract.* In these experiments the blood sera of a bronchial asthmatic sensitive to horse dander, and of guinea pigs sensitized to horse dander by inhalation, were passively transferred to the normal human skin and to normal guinea pigs. Positive passive transfers were obtained both from the transfer of the human serum and of the guinea pig serum. This criss-crossing of the human reagin and of the anaphylactic antibody suggests that both antibodies are identical.

### *Discussion*

(Dr. A. F. Coca, New York City.) It seems much too strong to say that the results obtained by Dr. Ella F. Grove and myself have been refuted by Cooke and Spain. We have independently tested the same sera that Cooke and Spain did (in fact, they obtained all of their sera from us), and we have not been able to obtain this passive transfer to the human skin in order to study it. Also we have given one serum, with which Cooke and Spain obtained positive results, to Dr. M. Walzer, and he has not been able in a single instance to see even a suggested passive sensitization of the human skin. We know that anaphylactic sera will sensitize guinea pigs uniformly, depending on their established sensitizing power. If we find several highly-powered anaphylactic sera which will sensitize guinea pigs regularly, but the human skin very inconstantly, we must conclude that whatever is active in the irregular skin sensitization in these sera cannot be the anaphylactic antibody. On the other hand, the few reported successes in sensitizing guinea pigs with the serum of human asthmatics have been so inconstant that they cannot possibly be referred to the reagents in those sera which will sensitize the human skin constantly, and in a certain dosage with the greatest uniformity. The results are so irregular on both sides that we cannot possibly think these two phenomena can be due to the same substances.

(Dr. Ratner, closing.) I agree with Dr. Coca that the accomplishment of the transfer from the human to the animal, and from the animal to the human skin is rather difficult, and for that reason I believe that a great deal of work should be continued along this line. It is conceivable to imagine that injecting one minim of an animal's serum into a human skin might not have enough of the sensitizing substance to be transferred to such an individual, that particular person being a normally resistant individual. The passive transfer of anaphylaxis from one animal to another is not uniform at all times. We have one instance where one of the animals gave birth to offspring and the offspring were definitely passively sensitized, and we could not take this mother's blood and

Among the human cases of illness there were eleven who had had contact with ill parrots and who finally were considered as having psittacosis. Cultural and serological tests showed no evidence of infection by *B. psittacosis*. The agglutination tests were repeated up to one month after the onset of the disease. These findings seemed definitely to exclude *B. psittacosis* as the etiologic agent of the disease.

Fortunately in one instance there became available an ill parrot and two patients who had come in contact with the parrot. Inoculation of parrots with materials from these three cases caused disease and death, and material from these parrots likewise caused death when inoculated into other parrots. The organ emulsions, when filtered through Berkefeld V filters, likewise caused disease and death. Two successive filter passages with the parrot strain and with one of the human strains were successful. These results and the failure to find any common bacterial factor seemed definitely to indicate that the disease, psittacosis, was due to a filterable virus. Mice were found to be susceptible to the virus.

As this demonstration was completed, Bedson, Western and Simpson (London) reported similar findings. Since then, Levinthal (Berlin), Armstrong, McCoy and Branham (Washington) have also demonstrated the presence of a filterable virus.

#### *Discussion*

(Dr. F. P. Gay, New York City.) I should like to ask Dr. Krumwiede if he has made any observations on chlamydozoa or other bodies of that sort in either animals or human beings.

(Dr. S. E. Branham, Washington.) I should like to emphasize what Dr. Krumwiede said about the absence of Nocard's bacillus. At the Hygienic Laboratory we examined bacteriologically at least twelve birds that had spontaneous psittacosis infection, twelve normal birds, twelve birds experimentally infected, and material from four cases of psittacosis, one fatal, and we did not find, although we picked more than 400 colonies, one single culture of any member of the paratyphoid group of bacteria.

(Dr. R. D. Lillie, Washington.) I have heard Dr. Krumwiede's paper with much interest, and I should like to mention some other findings we have observed in the Hygienic Laboratory which are a little bit aside from the filtrations. I had an opportunity of examining histological tissues on some fourteen parrots and parrakeets, three of which showed lesions. One of these was directly associated with human cases of the disease, and the rest were intimately exposed to material from other parrots that were definitely associated with human cases. These three parrots showed focal coagulation necroses in the liver, and large Kupffer cells, and in one of them there was a proliferative reaction of the pleural endothelium. In all of the three parrots I found very minute coccoid and bipolar bacillary cell inclusions in the Kupffer cells of the liver, in the pleural endothelial cells, and also in the bone marrow. These findings are parallel with those from two human autopsies which I had studied through the kindness of the Department of Health of Philadelphia and of the Naval Medical School in Washington. These human cases also showed the focal necroses in the liver. The major lesion was in the lungs, where there was pneumonia with the exudate largely of large round celled type, and in these two cases I was able to demonstrate after prolonged search the same inclusions in these large round cells, both within the exudate and within the alveolar wall. These cell inclusions measure between 0.2 and 0.3 micron, and I believe they

PENETRATION OF ANTIBODIES IN THE CENTRAL NERVOUS SYSTEM. Jules Freund, Philadelphia, Pa.

*Abstract.* Antibodies can be extracted from the brain and spinal cord of rabbits actively or passively immunized with typhoid bacilli.

The titers of the antibodies in the extracts of brain and cord depend upon the titer of the blood serum. In actively immunized rabbits the following numerical relationships exist between the titers of the serum and of these organ extracts: The ration of the titer of the serum is to the titers of extract of brain and of the spinal cord about as 100 is to 0.8; the titer of the serum is to the titer of the cerebrospinal fluid as 100 is to 0.3. In passively immunized rabbits the titer of the serum is to the titer of brain and spinal cord extract as 100 is to 0.7.

The antibodies recovered from the brain are not due to the presence of blood in it, for perfusion of the brain does not reduce its antibody content appreciably.

Antibodies penetrate into the spinal fluid from the blood even in the absence of inflammation of the meninges. When the penetration is completed, the following numerical relationship exists between the titer of the serum and that of the cerebrospinal fluid: 100: 0.25.

The penetration into the cerebrospinal fluid of antibodies injected intravenously proceeds at a slow rate, being completed only several hours after the immune serum has been injected. The penetration of antibodies into the tissue of the brain occurs at a very rapid rate. It is completed within fifteen minutes.

It is very unlikely that when the immune serum is injected intravenously the antibodies reach the brain tissue by way of the cerebrospinal fluid, or that the antibody titer of the cerebrospinal fluid is lower than that of the brain extract, and that antibodies penetrate faster into the tissue of the brain than into the cerebrospinal fluid.

#### *Discussion*

(Dr. M. B. Lurie, Philadelphia.) I should like to ask what implications follow from the studies on the distribution of antibodies in the brain and blood of rabbits, in regard to the mode of circulation and formation of cerebrospinal fluid.

(Dr. Freund, closing.) Following intravenous administration of immune serum, antibodies accumulate first in the brain and cord and only later in the spinal fluid. There are more antibodies per gram of organ in the brain and cord than in the spinal fluid (per cc.). On this ground I believe that antibodies found in the brain and cord reach these organs through their blood vessels and not by way of spinal fluid.

Of course these observations have no direct bearing on the question of what would happen if the serum were injected into the lumbar space. Work on this problem is in progress.

ETIOLOGY OF PSITTACOSIS. Charles Krumwiede and (by invitation) Mary McGrath and Carolyn Oldenbusch, New York City.

*Abstract.* About Jan. 15, 1930, the Department of Health became cognizant that there was illness among persons in contact with sick parrots. The first consideration naturally was whether or not *B. psittacosis* was causing this disease. Of a total of twenty-nine sick or dead parrots received, only one showed the presence of *B. psittacosis*. This parrot had died in a pet shop, and none of the human beings who came in contact with it developed any signs of illness.

It was found that cells from bone marrow and spleen of tuberculous guinea pigs when planted in plasma from tuberculous or non-tuberculous guinea pigs fail to migrate and to multiply in concentrations of tuberculin prepared from either the human or the bovine type of tubercle bacillus, and on the other hand that such concentrations of tuberculin do not interfere with the migration and multiplication of cells from bone marrow or spleen of non-tuberculous guinea pigs. When high dilutions of tuberculin are added to explants of bone marrow or spleen of tuberculous guinea pigs, migration of cells occurs, but the cells are shrunken and rounded, whereas the cells of tissues from the non-tuberculous animals are larger, irregular in outline and vesicular.

Tuberculins prepared from the avian type of tubercle bacillus, from the various acid-fast bacilli found in fish, snakes, frogs and turtles, from several different strains of the lepra bacillus and from a number of different acid-fast saprophytes did not interfere with the migration and multiplication of the cells from explants of bone marrow or spleen of tuberculous or of non-tuberculous guinea pigs. Similarly tuberculoprotein, the phosphatid and the carbohydrate fraction of the human type of tubercle bacillus fail to inhibit the migration and multiplication of the cells from the bone marrow or spleen of tuberculous or of non-tuberculous animals.

The addition of plasma from a tuberculous guinea pig to explants of the bone marrow or spleen of a non-tuberculous guinea pig does not render such explants susceptible to the cytotoxic action of tuberculin prepared from the bovine or human type of tubercle bacillus.

### *Discussion*

(Dr. E. R. Long, Chicago.) Tissue culture experiments on tissues removed from tuberculous animals which are sensitive to tuberculin, always raise this question: How far down the line of descent from the original sensitive cells does the sensitiveness persist? I may say that in the testes of the tuberculous animal, where we can see clearly the line of descent, the spermatocyte is profoundly sensitive to tuberculin, whereas its grandchild, the spermatozoön, is not demonstrably sensitive.

(Dr. Aronson, closing.) Fisher found that fibroblasts which had been grown from tuberculous chickens for several generations were sensitive to the action of avian tuberculin. In my studies I found that the bone marrow cells from the first generation of a tuberculous guinea pig became rounded and shrunken when tuberculin was added.

THE MECHANISM OF THE INFLUENCE WHICH AN INFECTIOUS PROCESS EXERTS ON THE IMMUNITY RESPONSE OF THE ORGANISM. L. Dienes, Asheville, N. C.

*(Abstract not received.)*

PROGRESS IN CHARACTERIZING ANTIBODIES AND ANTIBODY ACTION. Stuart Mudd, Balduin Lucké and (by invitation) Morton McCutcheon and Max Strumia, Philadelphia, Pa.

*Abstract.* Analysis of the mechanism by which blood serum promotes the phagocytosis of bacteria and other foreign particles has been continued. Sera which promote phagocytosis of bacteria or of red blood cells have been found invariably to cause certain definite changes in the surface properties of their homologous antigens, namely, increased cohesiveness, decreased surface potential

would be able to pass a filter, if any visible organisms can be said to pass filters (*cf.* U. S. Public Health Reports, 45, 773, April 11, 1930).

(Dr. Herbert Fox, Philadelphia.) Is there any evidence of difference in the infection of parrots of this group that has come from South America, and previous outbreaks of what has been called psittacosis as far back as the naming of the organisms in 1892? In the past twenty-four years at the Philadelphia Zoölogical Gardens there have been four outbreaks among parrots, to which the term psittacosis may be applied in the sense that there was a pneumonia, a degeneration and infiltration in the liver, and on two occasions, when the attempt was made, organisms of the paratyphoid-paracolon group were isolated. From what I can learn of the cases this time, the picture is not the same. Is there any evidence to indicate that the disease among birds is distinctly different from that in former years, because the psittacosis organism was not difficult to obtain from the previous outbreak?

(Dr. Stuart Mudd, Philadelphia.) There are several other visible micro-organisms which can be said surely to pass the filter under proper conditions.

(Dr. Krumwiede, closing.) As regards the disease in parrots, I have no doubt that there is a definite *B. psittacosis* infection in parrots, analogous to the infections we have in laboratory animals, in rodents, in food-producing animals (including ducks), and in man, as Miss Cooper and I, as well as others, have shown.

With the unfortunate illness of Miss McGrath, Miss Oldenbusch and Miss Buzen who assisted, our work was necessarily curtailed. The material available was turned over to Dr. Rivers as he wished to study the virus, also the histology, facilities for which were not immediately available to us.

In regard to Dr. Lillie's important observation on tiny bodies in the tissue, I might add that Levinthal (in a reprint just received) states he observed coccoid and bacillary forms in smears from tissues and filtrates.

#### A STUDY OF THE EFFECT OF TUBERCLE BACILLUS LIPOIDS ON THE TUBERCULIN REACTION. Esmond R. Long and (by invitation) Arthur J. Vorwald, Chicago, Ill.

*Abstract.* Doses of 0.02 to 0.05 of a milligram of purified soluble tuberculin protein cause an intense interstitial cellular infiltration and profound degeneration of the cells of the spermatic tubules in the testes of tuberculous guinea pigs (tuberculin reaction). Equal doses of purified lipoids in fine colloidal suspension cause a slight interstitial wandering cell infiltration and no degeneration. Mixing tubercle bacillus fatty acid with tuberculin protein enhances the tuberculin action of the latter in that the fluid element of the exudate is greater and the amount of fibrin present is increased. No change in the intensity of cellular infiltration or necrotizing effect on the testicular tubules or spermatocytes has been noted. The increased fluid output suggests that an increased degree of toxic action on the capillary endothelium has occurred. Ultramicroscopic examination has shown no change in the degree of dispersion of the colloidal particles incident to mixing the two colloidal preparations.

#### THE SPECIFIC CYTOTOXIC ACTION OF TUBERCULIN ON TISSUE CULTURE. Joseph D. Aronson, Philadelphia, Pa.

*Abstract.* The specific cytotoxic action of tuberculins prepared from the various acid-fast bacteria upon the bone marrow and the spleen of tuberculous and of non-tuberculous guinea pigs was studied by means of tissue culture.



precipitated by dilution and acidification has an iso-electric point at pH 5.1, whereas the precipitin, uniting with the antigen, gives a specific precipitate whose iso-electric point is at pH 5.7 to 5.8. The differences between these two values may be due to altered orientation of the antibody, possibly some of the dissociating groups being combined, or else one of several other possibilities must obtain. We are investigating these questions further.

In regard to Dr. Freund's question, I may say that the difference between the minimum solubility of pH 6.6 and 6.8 which Felton found for his anti-pneumococcus antibodies, and our value of pH 5.6 to 5.8 does offer a very important point of departure for further analysis of what this antibody protein is. Felton and I have been in communication, and intend to go ahead and begin further analysis of this point in the next few days, but I have no other data to offer at the present time.

THE BUCHNER RENAISSANCE IN IMMUNOLOGY. W. H. Manwaring, Stanford University, Calif.

*Abstract.* There is convincing evidence that injected antigens undergo a series of biochemical "hybridizations" in animal tissues, and suggestive evidence that the resulting antigen-"hybrids" become semipermanently "symbiotic" with these tissues, both terms, of course, being used metaphorically.

There is convincing evidence that some of these antigen-"hybrids" have properties simulating those of specific antibodies, but no proof thus far that they are identical with these antibodies.

The "hybridization" metaphor, however, furnishes a logical and consistent explanation of all known facts of specific immunological adaptation, and is the nearest approach to a complete immunological theory that can be formulated at the present time.

There is no proof at the present time, however, that antigen "hybridization" is the only mechanism of specific immunological adaptation, and no suggestion that such "hybridizations" are not supplemented by non-specific functional mutations and hypertrophies.

### *Discussion*

(Dr. F. P. Gay, New York City.) I should like to speak with enthusiasm of this work of Dr. Manwaring's, as I made experiments in 1912, embodied in two typewritten pages of manuscript which was never published, because I could not repeat the results. I made a mixture of normal horse and rabbit serum, and incubated it, and in one instance I found precipitins on adding horse serum to the mixture, but I was never able to repeat it, and I am glad to find I was probably right, after all.

(Dr. F. M. Huntoon, Glenolden.) I should like to add to Dr. Gay's experience. Once by incubating an extract of pneumococcus Type I with the juice obtained from cabbage, I was able to say later that this mixture contained agglutinins for the pneumococcus. Later I showed that it contained protective antibodies also. Like Dr. Gay, I never published my results, because I spent three months trying to repeat them, and could not do so.

(Dr. Stuart Mudd, Philadelphia.) I think it is only fair to Dr. Arthur Locke to say that he has done the same thing by incubating red blood cells with serum, and by treatment with ether, splitting off hemolysins, and he has published his results.

difference and increased resistance to wetting by oil. Similarly, but with certain exceptions, sera which cause these characteristic changes in the antigen surfaces also promote phagocytosis. The phagocytosis-promoting, agglutinating and surface effects are in quantitative correspondence. A similar striking correspondence has been found between the agglutinating, surface and phagocytosis-promoting effects caused by the euglobulin and pseudoglobulin fractions of antibacterial sera.

Following the technique of F. S. Jones, collodion particles were coated with precipitinogen and then treated with homologous precipitin sera and their protein fractions. The same sera and serum globulin fractions cause specific precipitation, agglutination or phagocytosis according to the conditions under which they are allowed to react with their homologous antigens.

Agglutinating, surface and phagocytosis-promoting effects have thus been found to be quantitatively parallel. This quantitative correspondence of the several effects of a serum or serum globulin fraction has been found both for antibacterial and for antiprotein sera and their globulin fractions. All of the effects are consequences of the deposit of sensitizing serum substances on the antigen surface. The surface deposit so produced by maximal sensitization with homologous rabbit antiserum has similar properties whether deposited upon acid-fast bacteria, upon erythrocytes, or upon precipitinogen-coated collodion particles, *i. e.*, *the sensitized surface has wetting properties characteristic of protein, is cohesive, and has an iso-electric point between pH 5.5. and 5.8. These are the properties also found for specific (immune) precipitate.*

These studies thus bring evidence of a new sort in support of the following simple generalization: *The combination of antigen and antibody is determined by specific chemical affinities. The effects following this combination, namely, precipitation, agglutination, changes in surface properties and phagocytosis, are consequences of the properties primarily of the antibody-protein combined with and deposited upon the antigen surface.*

### Discussion

(Dr. C. B. Coulter, New York City.) The point which Dr. Mudd has emphasized with regard to the shift of the iso-electric point toward the more alkaline region is I think a rather important one. There are plenty of people who would say that the immune body is a perfectly independent substance and not an essential part of the protein with which it is associated. If that were the case, we should not expect the shift which Dr. Mudd has described. The explanation which was discussed by Dr. Mudd seems to be not wholly satisfactory, that is, the presence of fibrinogen which has itself a more alkaline iso-electric point than that of the euglobulin cannot be the explanation. The data of Dr. Mudd suggest that the immune element of the protein molecule may involve the properties of the molecule as a whole; this is perhaps reflected in the *orientation* of the protein molecule in the process of combining with antigen in the immune reaction.

(Dr. Jules Freund, Philadelphia.) Is it possible to isolate from immune serum obtained from the rabbit a substance that has an iso-electric point of pH 5.7? Dr. Felton isolated a very potent substance from immune horse serum, which has an iso-electric point (minimum solubility) at pH 6.6.

(Dr. Mudd, closing.) I can only agree with Dr. Coulter's remarks. There is one item of evidence I did not stress which points in the direction he has indicated, namely, that the euglobulin, which is one of the active fractions, when

## Discussion

(Dr. A. S. Warthin, Ann Arbor.) About six years ago I found in the crypts of two tonsils that came in the ordinary diagnostic work in my laboratory, organisms similar to these. At that time I regarded them as simply spore cases of some unknown mold, but since then our attention has been drawn to the *Rhinosporidium*. I believe that they represent *Rhinosporidium*. Dr. Weller thinks it is probably a new species. I sent photomicrographs of these two tonsils to Dr. Talliaferro in Chicago, and he regarded them as *Rhinosporidium seeberi*, but asked to see the sections, which I have not sent him as yet. If they prove to be *Rhinosporidium seeberi* these will make the fourth and fifth cases on record in North America. It is interesting that one of these cases was a young female, which would be the first case in which a female was infected.

CELLULAR IMMUNITY IN ACQUIRED AVIAN MALARIA. P. R. Cannon and (by invitation) W. H. Talliaferro and L. G. Talliaferro, Chicago, Ill.

*Abstract.* Previous papers in this series by Talliaferro and Talliaferro demonstrated a definite immunity to superinfection in canaries infected with *Plasmodium cathemerium* and indicated that this immunity is not associated with a humoral antibody, inasmuch as serum from birds with latent malarial infections was without both protective and curative action on the development of the parasites in normal birds.

The present paper reports observations on the cellular reactions in the tissues of normal birds and of others superinfected. The tissues were fixed, sectioned and stained with Maximow's hematoxylin-eosin azur II. In the normal birds it was found that phagocytosis begins as soon as parasitized red blood cells are injected, being most pronounced in the spleen and liver, and consisting in the ingestion of the parasite-red cell combination rather than of individual merozoites. In spite of a constant rate of phagocytosis, many parasitized red blood cells escape ingestion and sporulation occurs with extension of the infection. Thus, the process advances until a crisis occurs on approximately the eighth day, after which time the rate of phagocytosis apparently exceeds the rate of reproduction and the infection becomes latent.

In the superinfection, however, the parasitized red blood cells injected are removed from the circulating blood stream extremely rapidly, so that within twenty-four hours there are few survivors to sporulate. This altered reactivity seems to be due to an actually increased number of actively phagocytic cells within the spleen and to a less degree in the liver. Because of this fact, the parasitized red cells are quickly removed from the blood and the superinfection terminated. The mechanism of the immunity then, is an increased rate of phagocytosis by the cells of the reticulo-endothelial system, resulting from an increased number of actively functioning phagocytic cells which are present in birds with latent infections.

THE PROTECTIVE EFFECT OF SPLENIC TRANSPLANTS IN ALBINO RATS AGAINST *BARTONELLA MURIS* ANEMIA. David Perla and (by invitation) J. Marmorston-Gottesman, New York City.

*Abstract.* *Bartonella muris* anemia is an infectious disease occurring in albino rats following the removal of the spleen. It may be transmitted into normal young rats and young rabbits by the injection of whole blood from anemic

(Dr. A. F. Coca, New York City.) The theory that antibodies are composed of antigen united with some normal body substance is attractive; however, the theory seems to face serious difficulty because of the long period of time during which antibodies are often produced after inconsiderable antigen injections. It is a well known fact that antibody production can continue, and often does continue throughout life after only a very moderate contact with the antigen, as in the case of antitoxins in what we call the naturally immune person. This could be explained, in a way, under the theory, by assuming a continual contact with the antigen throughout life after the antitoxin has once been formed, but Park's observations seem to have settled that argument very conclusively when he showed that an attempt at active immunization in babies who were still immune because of the transfer of passive immunity from the mother to the child, did not, after a short period, show an acquired active immunity. Just as many of these children become susceptible after this treatment as would have been expected if they had not received the injections. In other words, the antigen contact in the presence of antibodies is not sufficient, under these conditions, to continue the antigenic stimulation. Therefore we still have to explain why antibodies continue to be produced throughout life. Also, we have the facts brought out by Heinbecker's study of the Eskimos, who presumably do not come in contact with diphtheria bacilli at all, and still show an immunity to diphtheria.

These considerations point to antibody production as the secretion of a synthetic body similar to the secretion of enzymes, rather than as an elimination of antigen conjugated with a normal body substance.

THE CYTOCHROME OF BACTERIA. C. B. Coulter (by invitation) and F. Stone (by invitation), New York City.

*Abstract.* The intracellular pigment cytochrome which is found in all living plant and animal cells except the anaerobic bacteria, was originally observed by McMunn, and was rediscovered and renamed by Keilin. We have studied the occurrence of this pigment, particularly in the corynebacterium group of bacteria. The four absorption bands observed by McMunn and Keilin are found by spectroscopic examination in suspensions of living bacteria of all the members of the group. In addition there is found a band lying in the red portion of the spectrum. Filtrates of cultures show no selective absorption except in the case of toxin-producing diphtheria bacilli, and in filtrates of these the intensity of selective absorption runs parallel with the amount of toxin present. The characteristic absorption spectrum may, therefore, be that of the toxin itself. It has been possible by spectroscopic examination alone of different filtrates, to determine the presence and approximate amount of diphtheria toxin.

RHINOSPORIDIUM SEEBERI: PATHOLOGY AND REPORT OF THIRD NORTH AMERICAN CASE. Carl V. Weller, Ann Arbor, Mich.

*Abstract.* The clinical findings and histopathology from the third case of *Rhinosporidium seeberi* to be reported from North America are described. The stages of the development of the parasite, as well as the character of the accompanying granulation tissue, are illustrated, as far as is possible. The foreign body giant cell reaction which succeeds the rupture of the old cysts is described for the first time.

tion due to the participation of the neutrophile. The presence of "myeloid" giant cells, which are megalokaryocytes, in the spleen, liver and lungs, suggests a similarity between the process and Hodgkin's disease.

The daily leucocyte records of the animals show a close parallelism with the histopathology found. In practically every instance the leucocyte picture became septic in type a short time before death occurred.

A group of rabbits inoculated six months previously with living human tubercle bacilli were given an intravenous injection of avian tubercle bacilli, the same amount being given to a group of controls on the same diet. The injection of human tubercle bacilli had caused a local abscess which ruptured and healed, as did the subcutaneous inoculation of avian tubercle bacilli. The animals had normal blood counts and were in excellent physical condition at the time of their avian inoculation.

One of these rabbits died within seven days. The remaining animals lived from four to five months. These animals showed marked involvement of kidneys and joints. There were a few tuberculous foci in the lungs and liver. The histopathology was similar to that seen in chronic tuberculosis in human beings.

Daily leucocyte records showed the establishment of a septic leucocyte picture long before death of the animal. This corresponds closely with the leucocyte picture present in chronic progressive human tuberculosis. Here again the leucocyte picture closely parallels the histopathology present.

**THE RELATIVE PHAGOCYTIC ABILITY OF MONOCYTES AND LEUCOCYTES.** Balduin Lucké, Max Strumia (by invitation), Morton McCutcheon (by invitation), and Stuart Mudd, Philadelphia, Pa.

*(Abstract not received.)*

**THE USE AND THE REASONS FOR THE USE OF THIOCRESOL TO STIMULATE WOUND HEALING.** Stanley P. Reimann, Philadelphia, Pa.

*Abstract.* Since Hammett has shown that the sulphydryl group ( $-SH$ ) is essential for cell division, this group, when presented in proper concentration to cells, should stimulate mitosis. The healing of wounds is essentially a matter of cell proliferation; consequently  $-SH$  compounds should hasten healing. The question to which group the  $-SH$  radicle should be attached is apparently not of essential importance although it is of practical interest. Of a number of compounds tried, thiocresol proved quite advantageous. Theoretically at least, when this compound is split, the small amount of cresol present tends to inhibit bacterial growth, while not interfering materially with mitosis of the tissue cells. On old wounds, ulcers, and so on, it is used as a wet dressing in a 1:10,000 solution. Certain practical points must be observed, such as the maintenance of proper growth balance between epithelium and granulations, etc.

**EXPERIMENTAL STUDIES IN POLIOMYELITIS.** Richard Thompson (by invitation), New York City.

*Abstract.* Efforts to adapt the virus of poliomyelitis to the rabbit organism and to produce poliomyelitis in rabbits by testicular injection, and by brain injection after testicular passage, produced no evidence that the virus could be adapted in this manner. Suggestive symptoms produced in very young rabbits were duplicated in non-specifically treated and in uninoculated controls. The admixture of a vaccine virus adapted to the rabbit organism, with the polio-

splenectomized rats and the strain of the virus maintained by repeated passage in young rats.

We have succeeded in isolating a strain of *Bartonella muris* anemia from normal non-splenectomized adult rats by injecting the blood of the anemic rabbit into young rats. This demonstrates the fact that the adult rat is a carrier of the virus *Bartonella muris* anemia. It is found further that splenectomy in suckling rats is not followed by the *Bartonella muris* anemia since during the suckling period the rat is not a carrier of the virus.

*Trypanosoma lewisi* infection in adult normal rats is accompanied by *Bartonella muris* anemia. The trypanosome infection produces profound changes in the pulp tissue of the spleen. Functional injury to this pulp tissue produces the same effect in the adult albino rat as splenectomy, in lowering the resistance to the *Bartonella* infection.

Less than one-fourth of the spleen left in situ is sufficient to prevent the development of the *Bartonella muris* anemia. Autoplastic transplants of small pieces of splenic tissue made six weeks prior to the removal of the spleen prevent the development of *Bartonella* anemia in over 50 per cent of the rats, whereas transplants made four weeks prior to splenectomy do not protect. Histological examination of the transplants in the animals that were protected from the anemia reveals complete regeneration of all elements of the splenic tissue, both malpighian bodies and pulp cells. In the rats that were unprotected, the splenic transplants showed complete regeneration of the malpighian bodies, but exhaustion destruction of the pulp tissue. This demonstrates that the pulp cells of the spleen are specific in the protective mechanism of the rat to *Bartonella muris* anemia.

#### Discussion

(Dr. P. R. Cannon, Chicago.) In studying this question a year or so ago, we made somewhat similar observations. In our experience we found that the only rats that did not have this infection were from the Wistar strain. In studying the spleens of these animals and the ones that had the *Bartonella* infection, the difference was clearly one of increased amount of phagocytic tissue in the animals with the *Bartonella* virus, with large splenic follicles rich in mitotic figures in the case of the infected animals, whereas the lymphoid follicles were compact and small in the animals free from infection. We had a similar experience in removing a large proportion of the spleen. As much as three-fourths of the spleen was removed with no anemia resulting; two weeks later, however, the removal of the remaining fourth was followed by the typical so-called *Bartonella* anemia.

#### AVIAN TUBERCULOSIS IN NORMAL AND VACCINATED RABBITS. E. M. Medlar, Mt. McGregor, N. Y.

*Abstract.* Virulent avian tubercle bacilli when inoculated subcutaneously into rabbits cause a local abscess which ruptures and heals. The rabbits do not develop generalized tuberculosis and do not succumb to the subcutaneous inoculation.

Rabbits inoculated intravenously with 1 mg. or more of virulent avian tubercle bacilli always succumb to the infection in from two weeks to a month. A study of the histopathology of such animals shows extensive involvement of spleen, liver and bone marrow. The cell chiefly concerned in the reaction is the mononuclear leucocyte or monocyte. Many lesions show slight abscess forma-

Four or more weekly or biweekly injections of filtrate followed by injections of live organisms caused in addition to the above-mentioned degenerative lesions, acute glomerulitis, focal infiltrations of lymphocytes, especially in areas where previous tubular degeneration had occurred, and scattered patches of early fibrosis with compensatory dilatation of the tubules. In 15 per cent of the animals, glomerular nephritis with characteristic epithelial crescents, early fibrosis of tufts and pericapsular fibrosis were also observed. Preliminary alteration due to toxin apparently renders the glomeruli more susceptible to action of live organisms with a resulting production of chronic glomerular lesions.

THE RELATION OF THE TYPE OF RENAL EPITHELIAL REPAIR AND RENAL FUNCTION TO THE NUMBER AND TYPE OF CASTS IN THE URINE. Wm. DeB. MacNider, Chapel Hill, N. C.

*(Abstract not received.)*

CHRONIC FOCI OF INFECTION EXPERIMENTALLY PRODUCED. Virgil H. Moon, Philadelphia, Pa.

*Abstract.* The usual methods of animal inoculation are not suited to the study of chronic focal infections. They do not approximate the conditions present in human diseases. A new method for producing foci of infection in animals is reported.

Applicators of cotton are loosely wound on No. 20 or No. 22 rustless wire so that slight force will slip the swab from the wire. A trocar and canula are inserted into the region to be inoculated, the trocar is withdrawn, leaving the canula in place. The applicator is dipped into the material to be inoculated and is inserted through the canula. The swab is pushed quite beyond the end of the canula. Then, upon withdrawing the wire, the end of the canula pushes off the cotton into the tissue or body cavity. The canula is then withdrawn.

The method is a simplified procedure for implanting infection in a porous substance within the tissues. The foreign substance serves to maintain the infection against effective body resistance. The method is well suited for producing chronic foci in subcutaneous and intramuscular areas and in the body cavities. By exposing the viscera, infection may be implanted in the spleen, liver, gall bladder or other structures. In such instances smaller canulas and correspondingly smaller applicators are used.

Dogs, which are so resistant to ordinary inoculations that they are seldom used for the purpose, are easily infected by this method. When inoculated with streptococci, masses of infected granulation tissue 3 to 5 cm. in diameter develop in a few weeks. The inoculated organisms are regularly recovered from the inoculated focus and frequently from the substance of other organs. Streptococci have been recovered in pure culture six to ten months after implantation.

Only young dogs and rabbits have been used in these experiments. The pathological changes resulting from these chronic foci of infection include verrucose endocarditis with rheumatic nodules in the heart musculature, skeletal muscles and subcutaneous tissue, acute vegetative endocarditis with multiple infarctions, peri-arthritis, osteo-arthritis with anklyosis and deformity, nephritis of various forms, splenic atrophy and fibrosis, proliferative endarteritis and others.

myelitis virus in similar injections and passages, did not aid the adaptation. The virus of poliomyelitis, whether alone or mixed with vaccine virus, does not survive twenty-four hours in the rabbit testicle.

No neutralizing substances against poliomyelitis virus could be produced in rabbits by the repeated intraperitoneal and intradermal injection of poliomyelitis virus or of poliomyelitis-vaccinia virus mixtures.

Although attempts to infect monkeys by intrastomachic injections, after bile irritation of the mucosa, were entirely negative, evidence was obtained that repeated intrastomachic injections after bile irritation may produce an appreciable degree of immunity.

No evidence could be obtained that the cellular elements of the blood contain the virus in any greater proportion than the whole blood.

SPECIFIC AND SPECIAL INFLAMMATIONS OF THE NERVOUS SYSTEM. Simon Flexner, New York City.

*Abstract.* During the past twenty-five years, largely because of the extensive epidemics of meningitis, poliomyelitis, and encephalitis which have prevailed in that period, much attention has been directed to infections of the central nervous organs. At the present time, attention is being concentrated on encephalitis, of the epidemic form or of the variety to which the designation "post-vaccinal and allied conditions" is being applied. The important fact that herpes virus is capable of inducing encephalitis in rabbits, guinea pigs, rats, and mice, and occasionally in monkeys, has come to play a significant part in the supposed etiology of epidemic encephalitis.

The purpose of the present report is to draw attention to the multiple incitants of encephalitis, and hence to distinguish between encephalitis as a clinical-pathological condition and epidemic encephalitis, which appears to be a specific infectious disease. Just as there are multiple incitants for the clinico-anatomical condition to which the term "diphtheritis" is applied, and a specific incitant of the disease diphtheria, so, probably, there are also many sources of encephalitis and, probably, a single etiological source of epidemic encephalitis. If this view is correct, then the mere production of encephalitis in animals does not imply the experimental reproduction of epidemic encephalitis.

PRODUCTION OF GLOMERULONEPHRITIS IN RABBITS BY *STREPTOCOCCUS HEMOLYTICUS*. Maud L. Menten, Pittsburgh, Pa.

*Abstract.* Four strains of *Streptococcus hemolyticus*, two of which belonged to the *Streptococcus scarlatinae* group, were used. Intravenous injections of 0.5 to 1 cc. of filtrates of the above, produced degenerative changes in the endothelium of the tuft. A few of the glomeruli were pale and bloodless, but the majority were congested and the endothelium was vacuolated. Injections of 5 to 10 cc., or larger amounts of the toxin precipitated from the filtrate, caused widespread, marked degeneration of glomerular endothelium and granular degeneration of tubular epithelium accompanied by varying amounts of hemorrhage into the capsular spaces.

Repeated injections of the filtrate (0.5 to 1 cc.) resulted in destruction and disappearance of many secretory units. In a few cases as many as 50 per cent of the glomeruli had disappeared when the animal was killed. Remaining glomeruli showed swelling, congestion and vacuolation of the endothelium.

Repeated injections (1 cc.) of suspensions of live organisms gave similar results but of much less intensity.



the number of bacterial species isolated from this region was quite limited. From one to six varieties of organisms were cultured from the cervix in the present series of cases.

As stated in the previous report, again the intradermal tests gave variable results with bacterins prepared from apparently the same bacterial species when recovered from distant foci of the same patient. For example, a staphylococcus aureus derived from the cervix gave a three plus (+++) reaction, while one from the sputum gave a negative reaction; a staphylococcus albus from the cervix also gave a three plus (+++) reaction, while one from the nasopharynx gave a negative reaction. On the other hand, a close correspondence between the reactions often occurred from like organisms from distant foci. This interesting biological distinction was previously suggested as offering an especially refined method of studying varieties and subspecies of an organism in particular instances by means of the skin test in the hypersensitive patient.

#### REPORT OF THE LYMPHATIC TUMOR REGISTRY. George R. Callender, Washington, D. C.

*Abstract.* At the last report to this Society 100 cases had been contributed to the Lymphatic Tumor Registry of this Association, the period covered being approximately four years. In the past year sixty-three cases have been received, a rate of increase of 250 per cent. I wish to take this opportunity to thank the members of this Association for this increased support. The response to my plea is very gratifying, but the rate of receipt of these cases is still far below what it ought to be. Very few of the cases received during the past year have been cases in which the diagnosis was easy.

The greatest value can be reached from this Registry only if the maximum effort is made by those registering cases to follow the cases to their conclusion, or over a sufficient number of years to ascertain the character of the condition. I believe the difficulties in diagnosis and prognosis of this class of tumors are due to the fact that individuals and groups do not follow up their cases. I further believe that we shall be able to offer definite assistance in diagnosis when and only when we have a sufficient number of cases which have been so followed up as to determine their nature and the changes which occur during the course of the pathological processes.

Please make an additional effort to send in particularly your doubtful cases.

The accompanying table presents a summary of the first 150 cases in the Registry. This summary is not intended as a classification, but it does show the divisions found to apply to those cases so far received.

#### TUMOR REGISTRY, 1930

Lymphosarcoma	
No mobilization in blood.....	16
With leukemia (acute).....	1
Lymphoblastoma (confined to lymphatic structures)	
No mobilization in blood.....	0
With mobilization in blood.....	15
Leukemic.....	6
Reticulum cell sarcoma.....	22
(One case with lymphatic leukemia)	

FURTHER STUDIES ON THE CERVIX UTERI AS A FOCUS OF INFECTION IN CHRONIC ARTHRITIS. L. W. Famulener and (by invitation) Frederic J. Matthews, New York City.

*Abstract.* About six years ago we began our studies on chronic arthritis from the etiological and the therapeutic standpoint. The chronic inflammatory condition exhibited by this disease would indicate that it is probably induced by an irritant, possibly of infectious origin. As a basis for the investigation, it was assumed that various infectious agents might be responsible for the condition, and that their toxic products might induce a hypersensitivity of certain tissues or structures of the body. If this assumption were true, the specific hypersensitivity might be manifested by the skin when properly tested by the causative toxic agent. Therefore the problem was approached by making careful studies of the bacterial flora of all possible infectious foci in the patient. In each instance, autogenous vaccines were prepared from the organisms isolated from these sources, standardized, and by means of intradermal tests it was determined whether or not the patient showed a skin hypersensitivity to them. Those organisms which gave positive skin reactions were selected for therapeutic purposes in the treatment of the patient.

Among the foci of infection, the cervix has been suggested as of much importance in certain cases of chronic arthritis in the female where instances of the disease are relatively high. In a preliminary report submitted to this Society two years ago, this particular focus was considered, and the results of our observations on a series of approximately seventy-five consecutive cases were discussed. Cultures had been taken, under aseptic precautions, from the os and high up in the vagina, and planted in duplicate on blood agar slants, "hormone" agar, and dextrose broth. One set was grown aerobically and the other anaerobically. By that method, approximately 20 per cent of the cases yielded no bacterial growth from the cervical material. As that method of culturing had not proved satisfactory, the media which were used in the earlier studies were discontinued, and during the past two years, the Rosenow dextrose-brain-broth medium has been employed with much better results. In the present series of cases, in only one instance out of thirty-five, no growth developed from cervical material, and in no instance did we fail to get a growth from the vagina in thirty-eight cases. Therefore it has become the routine procedure to use this particular medium and no other in making cultures of materials from the cervix and vagina. It is especially valuable in the culturing of the streptococcus and the enterococcus groups. In this report the bacteriological findings from both the cervix and vagina are combined, as owing to their intimate anatomical relationship, much similarity occurs between their respective flora, although marked distinction between the two not infrequently occurs. The organisms isolated in the order of their frequency from these combined sources were as follows:

Staphylococcus albus (all types) .....	76 + %
Streptococcus (all types) .....	66 - %
Enterococcus (all types) .....	55 + %
B. coli (all types) .....	42 + %
Staphylococcus aureus (all types) .....	16 - %

Diphtheroid bacilli were occasionally found; the tetragenous in one case; Gram-negative coccus, one case; micrococcus albus type, one case. As noted,

definite histological changes. There were eleven patients who died from chronic diseases and no striking changes were found in the thyroid in this group.

It was stated that sections were taken from people of various ages, and a rather large group has been studied in the hope of finding a uniform histological picture for normal people of different ages. So far no conclusions have been drawn as to what is normal tissue for the different ages, and I must disagree with Hertzler when he states: "For practical purposes I am convinced that if one keeps in mind the various changes normal to the different periods of life as above indicated, the histology of the thyroid gland will be found to be fairly constant, rendering it unnecessary to assume that it undergoes changes unknown in other organs."

In studying patients with thyroid diseases, one is at times unable to place the case in one of the groups given for the classification of goiter, such as adolescent, adenomatous or exophthalmic goiter, and one frequently sees cases change from one type to another while under observation. From this histological study it would seem also that a diagnosis made purely on the histological picture without taking into consideration the clinical history and laboratory data is also open to error, and it seems that one will be forced to consider the different types of goiter merely as a stage of a continuous disease and not a definite clinical entity, as has been believed in the past.

### *Discussion*

(Dr. G. McC. Robson, Philadelphia.) This paper has interested me greatly. Dr. MacFarland and I have reported a study of a series of 100 thyroids secured at autopsy from individuals of various ages, dying of various diseases, acute and chronic (*Arch. Path.*, 1929, 7, 628-639). We found just what has been reported here — that there was no correlation between the structure of the thyroid and age, sex, color or morbid condition of the patient. The variations which we observed were even greater than those in the slides presented here.

PSEUDO TUBERCULOSIS OF THE THYROID GLAND. Plinn F. Morse, Detroit, Mich.

*Abstract.* The favorable prognosis which has heretofore been attached to so-called tuberculosis of the thyroid has been the subject of frequent comment by various writers. It has been known that although the thyroid gland operated upon for some type of goiter might show on microscopic examination large numbers of so-called tubercles, the patients usually run a favorable course and react in the usual manner to the thyroid operation, without later succumbing to tuberculous infection, either in the thyroid region or elsewhere.

The cases of so-called tuberculosis of the thyroid have usually failed to present tuberculous lesions in other parts of the body, and the postoperative search for other signs of tuberculous infection has been negative.

The cases that have been diagnosed at the Harper Hospital as tuberculosis of the thyroid have run a favorable course, and all of the patients are at present living and well, several years postoperative, with the exception of one patient who died seven years after operation from acute intestinal obstruction — cause unknown.

The histological study of the lesion seems to justify the assumption that probably the epithelioid reaction resembling tuberculosis is a result either of some other infection, or some metabolic disturbance which causes the colloid

Hodgkin's Disease		
Cellular.....	10	
Sclerosing.....	18	
Sarcomatous .....	12	
Mycosis fungoides (and Tb.) .....	1	
Lymphoblastic erythrodermia .....	1	102
	—	
Myelogenous leukemia.....	4	
Myeloma .....	1	
Chloroma .....	3	
Infectious mononucleosis (?).....	2	
Undiagnosed .....	14	
Lymphoepithelioma .....	5	
Carcinoma .....	12	
Inflammation (1 Tb.).....	7	48
		—
		150

### Discussion

(Dr. William Boyd, Winnipeg.) I should like to say that in the classification of these tumors there are remarkable differences of opinion. Just a week before leaving Winnipeg I received a report of a case which we sent to the Registry some time ago in which the initial lesion was in the nasopharynx; there were large glands in the neck, and the patient finally died. There were three opinions sent to me from the Registry. The first was that the case was a reticulum cell type of lymphosarcoma; the second, from another man, that it was a transitional cell carcinoma, and the third, from still another man, that it was a case of acute Hodgkin's disease. These three opinions came from men high up in this special field of pathology, but I was left very much in doubt about the case, and I should like to ask Dr. Callender how my case is to be classified.

(Dr. Callender, closing.) As we have no classification, the Registrar, who is really the clerk of the committee, makes a temporary decision which is based on the classification shown there this morning.

HISTOLOGICAL STUDIES OF THE THYROID GLAND. J. William Hinton (by invitation), New York City.

*Abstract.* Since the question frequently arises as to what constitutes normal and abnormal thyroid tissue, we have obtained for study sections from people whose deaths were due to accidental causes. These cases vary in age from stillbirths to 89 years. As a means of comparison there are also sections from people who died from acute and chronic diseases.

By Jan. 1, 1930, sections from 107 cases had been studied. It was intended to get sections from a large series of cases, rather than serial sections on a few cases, as these would represent more nearly the pathological reports that are received from operative specimens. So far there have been eighty cases of accidental death from which sections of the thyroid have been studied. There are also twenty-seven cases from patients dying from some systemic disease, and it was thought best to divide them into two groups: (1) cases of acute diseases, patients dying in less than three weeks from the onset of the disease; and (2) chronic diseases, those extending over months or years. There were sixteen deaths due to acute diseases, but the sections in this group failed to reveal any

and cell débris are spilled into the interstitial tissues. This phenomenon is usually regarded as terminal, but it is evident from a study of the literature and some twenty-five cases of thyroiditis in patients entering the University of California Hospital that minor infections or intoxications, perhaps even emotional shock and over-stimulation of the sympathetic system, may induce a similar degeneration of glandular elements. Since such patients do not die, there is an opportunity for a reaction to take place, and this is usually in the form of lymphoid cell infiltration or proliferation. Plasma cells and monocytes may be present, the latter sometimes in the form of giant cells. Pseudogiant-cells are formed by masses of colloid which contain the degenerate nuclei of epithelial cells.

The most constant picture is a rather diffuse desquamation of epithelium, the nuclei appearing in the previously contained colloid within the acini, and a diffuse infiltration with lymphocytes. These latter cells, as well as plasma cells, seem to arise in situ, as evidenced by the number of mitotic figures present in them. Stages in the degeneration of thyroid epithelium appear to be a swelling with fatty degeneration, desquamation and loss of nuclei, and later a breaking down of the substantia propria, so that agglomerations of cytoplasmic masses occur. These eventually disappear, but the process in some cases becomes progressive and prolonged even after (so far as can be determined) the initial stimulus has disappeared. This tendency to progression gives rise to the condition known as chronic thyroiditis, and with great overgrowth of lymphocytes and the concomitant proliferation of fibroblasts, the condition known as Riedl's struma may be the end-result.

Coincident with the degeneration of thyroid cells a more or less active regeneration usually takes place. This may be insufficient to take care of physiological demands, and so symptoms of hypofunction or even myxedema may occur. Or there may be a sufficient regeneration, and we get the histological picture of a moderately hyperplastic gland (associated with thyroiditis), but no symptoms of thyroid dysfunction. This regeneration may proceed to any degree, and a group of our cases exhibit the signs of thyroiditis (pain, pressure on the trachea, hoarseness, etc.) along with the vague symptoms of hyperfunction; and a last group show histologically a marked overregeneration and hyperplasia, associated with frank and definite symptoms of exophthalmic goiter. During this process of regeneration the lymphocytes gradually become less diffuse, become clumped, and in late stages definite lymph follicle formation occurs — the "lymphoid exhaustion" picture of Dr. Warthin. A further stage, conjectured only, would be a frank hyperplasia of the thyroid with characteristic lymphoid cell accumulations and without signs of thyroiditis or cellular degeneration. It would seem that a small proportion of typical thyroid hyperplasias might develop in this manner. It would be unwise, however, to infer that any considerable number pass through these stages.

### *Discussion*

(Dr. A. S. Warthin, Ann Arbor.) I should like to ask Dr. Connor about the administration of iodine in these cases. Most of them impress me as being examples of iodine treatment.

(Dr. E. T. Bell, Minneapolis.) I want to ask Dr. Connor what the relation of this exudative type of thyroiditis is to Riedel's struma, ligneous thyroiditis.

(Dr. Connor.) In answer to Dr. Warthin's question, there was never any

of the gland to assume the rôle of a foreign body. By a study of our cases here, I am impressed that the tubercle results as an effort to absorb and wall-off colloid which has presumably undergone some change which makes it a foreign body. On the basis of these arguments, I am inclined to think that the question of tuberculosis of the thyroid should be left open for further review, in that strong doubt is cast upon the assumption that the Koch bacillus is the etiological factor.

### *Discussion*

(Dr. A. S. Warthin, Ann Arbor.) I should like to ask Dr. Morse if any of these patients have been subjected to radiation. We have had a large number of patients with pseudotubercle of the thyroid of this nature in our material, and in all the cases except two, the thyroids had all been subjected to radiation. As a result of the irradiation, the acinar cells are necrosed, and the colloid acts as a foreign body excitant, and foreign body giant cells appear. While true tuberculosis of the thyroid is more common than one would suspect in the literature, these pseudotubercles are very common. I am quite sure in most cases they are the result of radiation.

(Dr. David Marine, New York City.) I should like to ask Dr. Morse whether he said guinea pig inoculations had been made, or not.

(Dr. Alfred Plaut, New York City.) When I read the title on the program, I expected to hear something about infection of the thyroid gland with the bacillus of pseudotuberculosis. The name "pseudotuberculosis" is meant for an infection with this bacillus. It would be better, for the sake of nomenclature, to call the lesions which Dr. Morse described, tubercle-like foreign body reactions in the thyroid gland.

(Dr. Ginsburg.) I should like to ask what were the clinical symptoms, and what were the diagnoses before operating on these patients.

(Dr. Morse, closing.) As to Dr. Warthin's question relative to the radiation, I do not know about that. That matter could be looked up from the histories, but it had not occurred to me.

As to the question of guinea pig inoculation, my point was that this is the weak place in our investigations. We have not done animal inoculations, and the nature of our material makes it impossible. In some laboratories where frozen sections are done routinely, such work might be productive.

I agree with Dr. Plaut that "pseudotuberculosis" is a bad term, and the condition should be called a tubercle-like foreign body reaction.

The clinical symptoms were those of the various adenomas of the thyroid, — the various forms of toxic thyroid that come into a routine thyroid clinic for operation.

**CHRONIC THYROIDITIS.** Charles L. Connor and (by invitation) H. H. Searls, San Francisco, Calif.

*Abstract.* The designation "thyroiditis" dates back to the early days of Kocher. His students, and surgeons in general, retain the name. There is some doubt, however, among pathologists as to whether such a condition actually exists. We wish to present a selected group of cases which, for want of a better name, have been classified as chronic toxic thyroiditis, and to illustrate a possible way in which they might develop.

It has long been known that acute infections and intoxications may cause degeneration of the thyroid epithelium and acini, and that as a result colloid

trols with normal thyroid showed 2.652 and 2.116 mg. Further controls were made with a carcinoma of the breast and an adenocarcinoma of the uterus. Both were negative. The figure 0.673 seems to be the highest for the iodine content of an ovarian struma ever obtained, and it seems high enough to help in asserting that these thyroid-like ovarian tumors can behave functionally in a way similar to thyroid gland. The fact that one specimen, in spite of looking exactly like the others under the microscope, contained no iodine whatever, is certainly important. No difference could be noted in the Mallory stain. Most of the examinations for iodine that have been reported in the literature do not reveal any iodine in the ovarian struma. In the face of the positive findings, however, it did not seem admissible to draw definite conclusions from the frequent absence of iodine. The microscopic pictures were not suggestive of embryonic thyroid tissue, which may be found free from iodine.

Some of the cystic cavities in the specimens contained material which looked different from the clear, brownish yellow thyroid secretion in the struma-like portions. This material gave no reaction for pseudomucin. None of the specimens contained structures which could be compared with a pseudomucinous or serous cyst adenoma. Glandular structures, as may be found in any ovary which is examined in many sections, were present. No statement can be made about their possible connection with the ovarian strumas.

The older literature on the subject deals mainly with the problem whether or not the thyroid-like tissue originated in the ovary itself. This question is settled. There remains the problem: Why does thyroid tissue occasionally grow to such large masses in the ovary? This problem is similar to the one concerning the predominance of the skin, teeth and bone in the dermoid cysts. The findings of Erwin Bauer that in his specimen the struma started from the surface epithelium, are obviously correct, judging from the pictures given in his paper. The literature deals mainly with the general conclusions Bauer has drawn from his serial sections and justly disapproves of them. But the fact remains that ovarian struma can originate from the surface epithelium. With the exception of Walthardt's three specimens, no complete serial sections are reported, and therefore nothing is known about the relations between surface epithelium of ovary and ovarian struma.

The frequent presence of ascites is clinically important, because generally ascites together with an ovarian tumor is taken as a sign of bad prognosis, while ovarian struma, in most instances, has proved to be benign.

#### BLOOD VESSEL INVASION IN ADENOMAS OF THE THYROID GLAND. Shields Warren, Boston, Mass.

*Abstract.* From 1923 to 1927 inclusive, thirty-four cases of adenomas of the thyroid showing definite invasion of blood vessels by clusters of tumor cells, but lacking any other evidence of malignancy, were found among the thyroid material received from the Lahey Clinic. None of these cases was suspected of malignancy clinically. Two cases showed recurrence, one in three months and one in ten months, and both patients died with multiple lung metastases, respectively ten months and two years after operation. The other thirty-two patients are living and well, from seven to two and a half years after operation, except for one patient dying of carcinoma of the uterus two and a half years after a thyroid operation. Thus, blood vessel invasion may be the only sign of malignancy in a small proportion of adenomas of the thyroid gland.

iodine given these patients, except the last few who had definite symptoms of hyperplasia. These few patients were given Lugol's solution; the others were not.

Riedel's struma apparently is a type of chronic thyroiditis, and some of these cases could have been called Riedel's struma.

(Dr. Warthin.) May I ask about the possibility of these patients having taken goiter cures? In numerous cases in which the thyroid presents evidence of the overuse of iodine, the physician has stated that the patient has had no iodine treatment, only to discover later that the patient had been taking a "goiter cure" containing iodine for many months.

(Dr. Connor, closing.) I cannot answer that; I do not know.

STRUMA OF OVARY. Alfred Plaut, New York City.

*Abstract.* Three specimens of ovarian struma (from three hospitals) were studied. The time elapsed since the operations was too short for definite clinical judgment. The ages of the patients were 47, 35, and 55 years of age. The gross aspect of the specimens added nothing new to the descriptions in the many reports in the literature. In all three specimens, other tissues were found besides the thyroid tissue, thus confirming the generally accepted opinion that ovarian struma is a teratoma thyreoideale. In two of the specimens all the tissues were benign; in the one from a woman 47 years of age, a large portion of the tumor was solid and gave a picture of carcinoma in the gross, as well as microscopically.

The thyroid character of the ovarian struma was obvious in all three specimens. The staining qualities of the contents of the follicles were typical. The three differently colored types of secretion were found with the staining method of E. J. Kraus. Papillary protrusions with high cylindrical epithelium and epithelial buds with high cells were present, giving the picture often seen in struma of a thyroid gland. It is possible to compare the structure of the malignant tumor in the one specimen with the rare type of trabecular adenoma of thyroid gland as described by Masson. The fact that this tumor was gelatinous did not preclude the possibility of its having a common origin with a thyroid-like structure. True mucin has been described in adenoma of thyroid by Nikolsky and by Wegelin. This possibly common origin is further suggested by the fact that mucin-producing solid tumor and typical thyroid struma are intimately mixed in large areas. And, furthermore, there are follicles in which the mucin and the typical thyroid secretion are found together. The ropy appearance of the mucin in the fixed tissue, together with the red color of the mucicarmine form a distinct contrast with the homogeneous, pale blue-staining thyroid secretion. It must be stated that the structure of the carcinoma-like tumor is similar also to some ovarian carcinomas, but the variety of ovarian carcinoma is so great that there is hardly any carcinoma which cannot be compared with one or another type of ovarian cancer.

All three specimens were examined for iodine. The method of Kendall and Richardson (*J. Biol. Chem.* 1920, 43, 161), was used. In one specimen, four analyses showed no iodine, while controls with normal thyroid gland gave figures from 1.70 to 1.88. In the second specimen 0.025 mg. of iodine were found per gram of dried ovarian struma. This figure compares with the figures of Robert Meyer (0.014 mg.) and of Neu (0.02 mg.). In the specimen which contained the carcinoma-like mass, the thyroid-like tissue and the solid carcinoma-like tissue were examined separately. Two analyses of the thyroid-like tissue showed 0.673 and 0.634 mg., while the solid, cancer-like tumor gave only 0.004. Con-



plasia of the thymus, and the other anatomical stigmas of the thymiclymphatic constitution. In addition, they present certain constitutional peculiarities of their own kind. Not all cases of thymiclymphatic constitution will present the Graves' disease syndrome, although all cases of the latter will possess the chief anatomical stigmas of this constitution. All forms of Basedowian or Graves' symptoms represent the abnormal reactions of a primary pathological anomaly. Basedowian or Graves' disease, "toxic goiter" and "toxic adenoma" are pathological reactions potentially predetermined in the individual at birth, by virtue of his constitutional anomaly. The potential Graves' constitution may be recognized histologically in the thyroids of very young children. To the underlying pathological and clinical entity of exophthalmic goiter, toxic goiter and toxic adenoma, I have applied the term "Graves' constitution."

### *Discussion*

(Dr. David Marine, New York City.) I quite agree with Dr. Warthin in regard to Graves' disease, that there is an anlage, a constitutional defect behind it. However, I cannot agree that they are all born with Graves' disease, and think the constitutional defect, which closely resembles that of status lymphaticus, can be acquired, and also can be congenital or hereditary. The acquired form develops around the menopause. It has something to do with insufficiency of the suprarenal cortex and the gonads. The older view spoke of Graves' disease complicated by status lymphaticus. I think that the lymphoblastic overgrowth is an integral part of Graves' disease. We can readily produce this lymphoblastic overgrowth by removing the suprarenal gland in any of the laboratory animals. Suprarenalectomy causes complete thymic regeneration in the rabbit and rat, and also hypertrophy of the lymphoid tissue in other parts of the body.

(Dr. H. L. Jaffé, New York City.) I should like to ask Dr. Warthin whether he found lymphoid hyperplasia in the thyroid in patients who were not toxic and were iodized.

(Dr. D. P. Seecof, Cleveland.) This is in line with Dr. Jaffé's question. In Colorado I was impressed by the large number of cases that had been iodized thoroughly, but on gross examination of the thyroid coming to the laboratory, I would diagnose it hyperplasia, because the thyroid was dense, grayish white, and showed no colloid. The picture was exactly as Dr. Warthin described it, and if it were not for the few groups of acini scattered through the gland, one would think he was dealing with a lymph node. But I cannot agree with Dr. Warthin regarding the difference in amount of lymphoid hyperplasia in the adenoma (the toxic adenoma) with or without symptoms. I could find no difference between the amount of lymphoid hyperplasia present in the adenomas from patients with, or without, symptoms.

(Dr. L. W. Smith, New York City.) The Lahey Clinic is very much interested in this question. In some 2000 cases of thyroid disease which we had the opportunity to section, we tabulated the cases, and grouped the lymphoid involvement on the bases of +, ++, +++, and +++++. In over 40 per cent of the cases of hyperthyroidism it is perfectly true one finds this lymphoid hyperplasia; in the remaining 60 per cent no hyperplasia is found. As this stimulated our interest, I took a series of normal thyroids, or at least thyroids removed routinely during the course of autopsies for other diseases, and in those instances we were able to find lymphoid hyperplasia throughout the thyroid gland in

**SKELETAL METASTASES IN CARCINOMA OF THE THYROID.** Isaac Levin, New York City.

*Abstract.* This study represents a continuation of several years of investigation of the pathogenesis of skeletal metastasis in carcinoma. The formation of these metastatic tumors in the bone, in the opinion of the author, depends not so much on the channels of transportation of the tumor emboli, as on the interaction between the proliferation of the transported tumor cells and the protective resistance of the adjoining normal tissue.

The significance of the thyroid in this study is due to the phenomenon that presumably benign tumors of the thyroid, and even normal thyroid tissue may form metastases.

From the clinical standpoint, the importance of the subject lies in the fact that the whole clinical symptom complex may be due to the skeletal metastatic tumors, while the primary carcinoma in the thyroid may cause no symptoms. In the cases reported, the condition in the bone was considered clinically to be primary, and the diagnosis was made only on the biopsy material.

**NOTES ON CERTAIN OF THE SO-CALLED SARCOMAS OF THE THYROID.** Lawrence W. Smith, New York City.

*(Abstract not received.)*

**THE THYMUS GLAND IN TOXIC AND NON-TOXIC GOITERS.** Alfred S. Giordano, South Bend, Ind.

*(Abstract not received.)*

**THE INTERACINAR EPITHELIUM OF THE THYROID GLAND.** Alan Richards Moritz, Cleveland, O.

*Abstract.* By means of silver impregnation of the periacinar reticulum and wax plate reconstruction of thyroid gland, it is shown that new acini are formed by intra-acinar and extra-acinar proliferation of epithelium. The extra-acinar proliferation is in the form of small solid or tubular buds which, when cut tangentially, may have the appearance of rests of undifferentiated interacinar epithelium. In hyperplastic areas of normal glands, and in glands the seat of pathological hypertrophy and hyperplasia, a labyrinthine intercommunication of acini exists.

### *Discussion*

(Dr. William Boyd, Winnipeg.) Three different schools of thought are represented in this paper. The first school stresses the importance of the interacinar epithelium. The more one studies these pathological thyroids, the more one is convinced of the budding outwards, as well as the inward budding of the acinar epithelium. I have some slides I would like to show taken from the lesion called fetal adenoma. I think one can see the budding in this type of lesion much better than in the hyperplastic thyroid of Graves' disease. In a section like this one can trace the proliferation of the lining cells with greater ease than in cases of Graves' disease. The two slides show the same picture, but are from different patients.

**THE SIGNIFICANCE OF THE LYMPHOID TISSUE IN EXOPHTHALMIC GOITERS AND SO-CALLED TOXIC ADENOMAS.** Aldred Scott Warthin, Ann Arbor, Mich.

*Abstract.* Exophthalmic goiter and "toxic adenoma" always present the pathological picture of hyperplasia of the primitive lymph nodes of the thyroid, hyper-

with colloid, if the iodine store is at least 0.2 mg. per gram of fresh tissue, and if the epithelium is low cuboidal, we have an adequate standard of normal.

2. *Hypertrophies and Hyperplasias*: All the work that has been done, whether with experimental regeneration following partial removal or the spontaneous or experimental production of goiter, shows that thyroid enlargement begins as a work hypertrophy and hyperplasia, and that in their developmental stages they are essentially the same for all animals. The sequence of events appears to be as follows: There is a decrease in iodine store, a decrease in the stainable colloid, an increase in the blood supply and a change in the epithelium from low cuboidal to high cuboidal and columnar. All gradations of this series of changes are observed from the earliest departure from normal to the extreme overgrowths of the marked hyperplasias.

Considerable confusion has arisen because pathologists have not always borne in mind the fact that progressive and regressive changes may succeed each other in rapid order and that the progressive and regressive changes are highly variable, both in degree and duration. Thus most goiters undergo hyperplasia and involution many times during their life history and each of these cycles entails certain morphological differences which may be misleading when too great emphasis is laid on any one detail. Thus, variations in the stroma, variations in the size of the follicle and colloid content alter the blood supply and possibly the nervous control which in turn modify secondary regeneration by giving rise to patchy or insular hyperplasia.

3. *Colloid Goiter*: Colloid goiter may be defined as the involution or as the return of an active hyperplasia to the condition nearest to normal, physiologically, chemically and anatomically, that a gland which has once been actively hyperplastic can assume. As is now well known, involution induced by iodine administration is identical with that occurring spontaneously. There is nothing degenerative or atrophic in the process of involution, although such glands may be the seat of highly degenerative or atrophic changes. Complete proof of this is established by the fact that colloid goiters regenerate as readily as normal glands following partial removal or when placed under a potent goitrogenic influence. The processes involved in the formation of a colloid goiter are the reverse of those involved in active regeneration or hyperplasia; that is, the blood supply decreases, the iodine store rises, the epithelium becomes cuboidal and the colloid increases in density. The development of a colloid goiter by the passive dilatation of normal thyroid follicles, I believe, is impossible.

4. *Exhaustion Atrophy or Sclerosis*: While involution to colloid goiter or physiological recovery is the usual mode of termination of compensatory hyperplasia, it occasionally happens, especially in endemic cretinism and in exophthalmic goiter, that the maximum degree of hyperplasia reached and the maximum capacity for function fail to bring about physiological compensation, and sooner or later a state of thyroid exhaustion supervenes, that is, atrophy in spite of attempts at regeneration. The essential changes appear to be analogous to those seen in atrophic cirrhosis of the liver; that is, they are the result of long continued hyperactivity and injury without sufficient physiological rest. In the first stages the appearance of such a thyroid differs very little from any other extreme degree of hyperplasia, save for the increase in stroma. Later, desquamation, atrophy and necrosis result. The cells become highly variable in size and staining intensity. The nuclei also become irregular, sometimes enlarged, hyperchromatic and sometimes pyknotic. The colloid is greatly reduced and as the process continues the follicles are reduced to nests of irregular cells in a dense stroma.

15 per cent of the cases, so while I agree with the major premise of Dr. Warthin that there may be a constitutional idiosyncrasy with respect to this lymphoid relationship and hyperthyroidism, I do not feel it is quite as sharp and clear-cut as he would indicate.

(Dr. Warthin, closing.) There are patients with Graves' disease who go all their lives without any symptoms. We would expect to find a certain number of cases showing lymphoid hyperplasia where the patient never develops exophthalmic goiter or toxic adenoma. Those cases which the gentleman from Boston mentioned are cases of quiescent Graves' disease, a potential Graves', but they may never develop it clinically. They have the possibility of having clinical Graves' disease, but they may never have it; as in the case where the patient went fifteen years, but still showed the lymphoid hyperplasia, and I am sure still has a big thymus. That is no argument against my theory, but only helps out my view.

In regard to Dr. Marine's statement of it being acquired, I do not believe the thymicolymphatic constitution is ever acquired. It is always congenital.

THE ESSENTIAL THYROID CHANGES IN GOITER. David Marine, New York City.

*Abstract.* Perhaps the greatest difficulty in understanding and classifying the essential changes in goiter has arisen from the fact that the thyroid is a very labile tissue, capable of rapid progressive, and even more rapid regressive, changes in all periods of life. In the life history of most goiters there are many of these cycles, and obviously, in a gland normally composed of retention cysts, each succeeding cycle becomes morphologically more complicated and more difficult of interpretation. This tissue lability and the long duration of goiter have led to the inclusion, as Virchow pointed out seventy years ago, of a great variety of secondary and terminal degenerative changes with the essential changes.

The clue for their separation was furnished by Baumann's discovery of iodine as a normal constituent of the thyroid. The extensive studies on the relation of the morphological changes to the iodine store, in both the naturally occurring and experimentally produced goiter which resulted from this discovery, have made it possible to work out a scheme of the sequence and relative importance of the major features of the thyroid cell and to separate the primary from the secondary changes.

Another cause of confusion has been the attempt to bring the morphological features into specific relationship with prevailing clinical classifications of human thyroid diseases, rather than to bring the clinical classifications into relation with the morphology, which is clearly a more constant and more natural standard. Thyroid hyperplasia is analogous to leucocytosis and has no more specific relation to metabolic diseases than leucocytosis has to infectious diseases.

Comparative studies have shown that the essential changes in goiter are similar in all the species of animals studied, and that they can be arranged in a comparatively simple scheme. When large series of human thyroids (autopsy) are studied in the light of the comparative pathology, the same cycle of changes is found so that one can arrange these morphological changes in a scheme that adequately presents the type and sequence of the changes which compromise the cell cycle in goiter.

1. *Normal:* The thyroid is so labile that to insist on too strict a definition of "normal" invites quibbling and defeats the object of establishing a practical standard. If the gland does not exceed 25 gm. in weight, if the follicles are filled

Observations were also made concerning the action of epinephrin and pituitrin on the blood vessels of the pancreas, including the circulation in the islands of Langerhans. Vasoconstrictor effects were noted with both drugs. In the higher orders of concentrations, blanching and cessation of the circulation in the islands were observed. With more dilute solutions, the effects upon the circulation in the islands were irregular and difficult to follow. Pituitrin was tolerated by the mice in much higher concentrations than epinephrin.

The results of the studies with epinephrin and pituitrin indicate that the circulation in the islands of Langerhans is probably regulated by changes in the afferent arterioles or the small arteries in response to physiological stimuli.

(a) THE SPLEEN IN SUBACUTE BACTERIAL ENDOCARDITIS OF THE VIRIDANS TYPE. (b) SYSTEMIC CLASSIFICATION OF SPLENIC PATHOLOGY. Herbert Fox, Philadelphia, Pa.

*Abstract.* (a) Despite the extensive literature on this clinical subject there are no general descriptions of the morbid anatomy of the spleen. This article summarizes the findings in twenty-five acceptably diagnosed cases. The main features of the results are as follows.

The most important gross lesion is the infarct. Practically every case shows perisplenitis, whether an infarct exists or not.

In early stages the organ is not greatly enlarged, but suggests a soft splenic tumor of the congestive type. In the later stages the organ is definitely enlarged, due to hyperplasia of its constituent tissue and to the inflammatory and hyperplastic changes incident to infarctions.

Hyperplasia of lymphatic elements proper is not a feature of this disease; on the contrary those tissues are inactive. Hyperplasia of cells of the reticulo-endothelial series is not seen early but appears more evident as the disease progresses.

Marked disease of the blood vessel lining is not an outstanding peculiarity of this disease. Evidences of blood destruction are missing in early, but present in older cases. Degenerations and coagulation within germ centers are frequently observed.

Structures suggesting Bracht-Wächter bodies have been seen several times. Clasmotocytes are occasionally seen but rarely in any number. Polynuclear neutrophiles are very prominent in spleens of this series of bacterial endocarditis cases. Eosinophiles are also frequently seen.

However, if we approach the scoring from the direction of the average change in each of the microscopic tissues, some light can be thrown upon the location in which the major changes are occurring. Instead then of making a sum of the score for each case, the clinicopathological groups are tabulated and their respective values averaged, the relative values for different diseases may become apparent. The group diagnosed as Banti's disease can be used as an example of the computation; they are listed in order of their duration.

60....	1	1	2	3	2	2	2	3	2	2	3	2	1
49....	3	1	2	3	3	2	2	3	2	2	2	2	1
3....	3	3	1	3	2	2	2	3	2	1	2	2	2
37....	1	1	2	3	2	2	2	3	2	3	2	1	1
56....	1	1	2	3	2	2	2	3	1	3	3	2	2
<hr/>													
	9	7	9	15	11	10	10	15	9	11	12	9	7
	1.8	1.4	1.8	3	2.2	2	2	3	1.8	2.2	2.4	1.8	1

5. *Struma Nodosa*: One of the secondary features of goiter in man requires especial notice, namely, the development of nodules of glandular tissue in long-standing human goiters, so-called adenomas. These growths have something in common with true tumor and also usually retain many of the physiological attributes of normal thyroid tissue. Knowledge of these growths is at present very inadequate. The fact that they are limited almost entirely to human goiters renders experimental approach difficult. That they are an integral part of diffuse goiter is shown by the fact that all nodular goiters, in their earlier stages, were diffuse goiters. This is shown in Wegelin's well known study. As to their origin, the idea of Woeffler that they arise from fetal rests, and the unfortunate term "fetal adenoma" introduced by Billroth have so influenced thyroid pathology during the last fifty years that it is difficult to undo the harm which this conception has introduced. While one cannot deny that occasionally congenital adenomas may arise from fetal rests, there can be no question that practically all of them arise from fully differentiated thyroid tissue and that their origin is intimately bound up with repeated hyperplasia and involution in long-standing goiters. This process, by altering the blood supply and nervous control, and through pressure effects, leads to insular, irregular and localized hyperplasias which eventually become so independent as to lose some of their physiological attributes and take on certain of the characteristics of tumor.

6. Lastly, I would like to call attention to the efforts Wegelin has been making to arrive at an acceptable international classification of the principal stages of goiter. If the principal features could be grouped in some acceptable way, the intermediate or subgroups could be left largely to individual fancy without endangering our ability to understand what is meant. It seems to me the following classification would include the essential major divisions in their sequential relations:

#### I. *Struma diffusa*

1. *Struma diffusa parenchymatosa*
2. *Struma diffusa parenchymatosa et colloides*
3. *Struma diffusa colloides*
4. *Struma diffusa sclerosa* (exhaustion atrophy)

#### II. *Struma nodosa* (adenomatosa)

1. *Struma nodosa parenchymatosa*.
2. *Struma nodosa parenchymatosa et colloides*
3. *Struma nodosa colloides*
4. *Struma nodosa sclerosa*

A STUDY OF THE ISLANDS OF LANGERHANS IN VIVO. Benjamin N. Berg, New York City.

*Abstract.* The islands of Langerhans were studied in the pancreas of the living white mouse under amytal anesthesia. Microscopically, they appeared as brilliant yellowish white bodies on the surface of the less refractile yellow pancreas, or attached to veins. Accurate observations were possible only when the islands were situated at the surface of the pancreas. Great variations were encountered with respect to the number of islands that were superficial.

In suitable preparations the circulation in the islands was distinguished clearly. The capillaries appeared as thin, thread-like loops that had U, S, V or spiral shapes and dipped into the substances of the glands. The circulation was extremely rapid and changes were noted in the capillary pattern. Rhythmic spontaneous contractions were observed in the small arteries.

The features were reduced from an original charting of 20 to 13, and this will have to be reduced still further. Sums of the numerical values of the characters do not permit distinct groupings of the spleens of the various forms of splenic anemia, nor do these scores differ sufficiently from those of purpura or bacterial endocarditis. In the limited group of viridans cases there is a rough separation of the spleens of acute and those of more protracted cases. Thus, the more acute cases range from 12 to 15, while the more chronic examples range from 14 to 19.

The only contribution this work can offer now is that an attempt is being made to schedule organic changes in the spleen in a numerical formula with the hope that some grouping will appear. The next steps are two—the formulation, around main characters, of the changes in less variable ones, and the comparison of numerical formulas assembled according to the clinicopathological diagnoses. Attempts to show that deductively, spleens will fall into groups that have a pathological significance have not been discarded. The features used are the following: follicles and germ centers, pulp and its splenocytes, sinuses and their endothelium, blood vessels, number and condition of wall, fibrous tissue, reticulo-endothelial cells, blood, pigment, neutrophiles and eosinophiles. Adventitious characters do not receive numerical values.

A [COMPARISON OF FOUR LINES OF MOUSE LEUKEMIA, TRANSMITTED BY INOCULATION. Maurice N. Richter and (by invitation) E. C. MacDowell, New York City and Cold Spring Harbor, N. Y.

*Abstract.* In previous communications we have reported the presence of an inbred strain of mice in which lymphatic leukemia occurs with great frequency, and the fact that the leukemias occurring spontaneously in this strain may be transmitted to normal young mice of the same strain by inoculation with emulsions of tissues.

From each of the spontaneous cases used as donors for the transmissions, a line of inoculable leukemia was obtained, transmissible apparently indefinitely by inoculation.

In these lines of experimental transmissions we have observed varieties of manifestation similar to those occurring in spontaneous cases. Some manifestations, however, have occurred so frequently in certain lines, and infrequently in others, as to indicate the presence of differences in the inocula prepared from each.

The transmissions of the several lines were carried on in parallel experiments, the host animals all being from the same inbred strain, and frequently from the same litter. Consistently reappearing differences in the lines are not, therefore, due to differences in the hosts.

### Discussion

(Dr. J. Furth, Philadelphia.) We have also succeeded in transmitting leukemia of mice. By intravenous inoculations leukemia is produced in a higher percentage of experimental animals than by intraperitoneal inoculations, and it may be transmitted even to a strain of mice in which spontaneous leukemia has not been observed. This necessitates some modification of the genetical theory of McDowell and Richter on susceptibility to transmitted leukemia. In about twenty-five days after the inoculation, about 20 per cent of the inoculated mice developed a systemic enlargement of the lymph nodes, followed a few days later by the involvement of the circulating blood, leukemia produced by intravenous

From these results the major tissue changes in Banti's disease are dilatation of sinuses and fibrous tissue overgrowth, well known features of the disease. But this emphasizes the inactivity of the splenic lymphoid elements and the reticulo-endothelial system. Pigment is shown to be increased. In the group of splenic anemia, secondary anemia and hematemesis, we find follicular and connective tissue, and reticulo-endothelial cells contributing to the splenomegaly.

In the hemolytic anemias, blood, reticular cell increase and fibrosis are the causes of enlargement. The purpura spleen has as its dominant change, sinus dilatation, bloodiness and reticular cell increase; on the whole it is an inactive spleen. In those cases in which the splenomegaly is the dominant feature of the case and there is no hepatomegaly, all parts of the spleen may be actively increased, but this is most marked by the high scoring of follicles, reticular cells, sinusoid structures and fibrous tissue. This is different from the cases in which enlargement of both organs exists and it seems to be independent of which organ enlarged first. The contrast is not great but is sufficient to note. In combined splenic and hepatic enlargement, blood vessel changes, sinus endothelial increase and fibrosis are much more marked than in the simple splenomegalies, which are for the most part inactive in lymphoid tissue, sinus endothelia are very prominent, reticular cells easy to find and fibrosis the highest scoring feature.

In the spleen with lymphocytosis, of which we have but two examples, there are lymph tissue atrophy, dilatation of sinuses, increase of fibrous tissue and richness of blood.

In splenomegaly accompanied by polynucleosis there are prominence of lymphoid tissue and excess of blood, while the pulp and its contents seem inactive.

The tissues contributing to the enlargement of the organ in subacute bacterial endocarditis are the sinus endothelium and the sinus size, blood and adventitious neutrophiles.

(b) The difficulty of giving a name or classifiable description to many spleens that are chronically enlarged, received from operation or autopsy, suggested the possibility of grouping them according to a system based on numerical values given arbitrarily to the principal anatomical features. The thought grew from a request made by a surgical colleague to score spleens as epithelioma is scored. The idea was first rejected as impossible but, an attempt being made, the writer found that a rough grouping did occur in two relatively easily separated groups, — one showing splenomegaly, hematemesis and fibrosis, and the other showing splenomegaly, purpura and pulp hyperplasia without lymph element participation. Mathematicians assured the writer that if the anatomical features adopted represented the correct selection and if the changes adopted were correct, it was acceptable to build formulas upon their digital arrangement or on their sum. This is by way of answering the question of the numerical value of a quality, because the changes in splenic pathology would be as much qualitative as quantitative.

A large chart was made, putting into columns all the gross characters of the whole organ and its section surface, all the characters seen under the microscope. If within normal limits they were evaluated at 2; if inactive or atrophic or less than normal their score was 1, and if larger, more active or hyperactive in anyway, the figure was 3. To this were added columns for adventitious changes such as infarct, which were graded 0 or 1. It was found shortly that scoring of whole organs or section surfaces led to no grouping whatever, whereupon scoring was confined to microscopic findings.



It was necessary to use relatively large doses of parathormone to produce fibrous changes in the bones of guinea pigs. The doses can be given to the guinea pig without producing fatal hypercalcemia, and the animal's ability to withstand such large doses may be related to the alkaline diet of this animal.

Even one dose of 60 units given to a guinea pig weighing 300 gm. produces extensive resorption, cessation of bone formation and infraction of the cortex within forty-eight hours. Such guinea pigs may die with extensive bone destruction, if the large doses are continued. We produced bone changes in guinea pigs with as little as 10 units of parathormone daily, and with 20 to 30 units daily for two or three weeks we obtained very extensive resorption and fibrosis of the bones.

In the dog, if the doses are large enough to produce rapid bone changes, fatal hypercalcemia may result. However, we have been able to prevent hypercalcemia in dogs on as much as 20 units of parathormone daily and have produced in these dogs generalized bone resorption with lacunar erosion, fibrosis, cysts and osteoid tissue — all criteria for the diagnosis of *ostitis fibrosa cystica*.

### *Discussion*

(Dr. Paul Klemperer, New York City.) I think this excellent paper is a very important contribution to decide the long debated question whether the parathyroid hyperplasia so often found in *osteitis fibrosa generalisata* is the cause or the result of the calcium deprivation of the skeleton. Previous experience, namely the observation of parathyroid hyperplasia in generalized bone carcinomatosis, has always caused me to consider the hyperplasia the result, and not the cause of the bone destruction, but I feel the presentation of Dr. Jaffé and his co-workers speaks entirely in the other direction. It is of course very important to decide this question, since surgeons here and abroad are starting to resect the hyperplastic parathyroid in order to cure the *osteitis fibrosa*. From the pictures presented, it seems as though Dr. Jaffé had been able to reproduce all the stages of *osteitis fibrosa*, both the hyperostotic as well as the hypostotic forms. I should like to know whether Dr. Jaffé has any ideas about the relationship of these experiments to the localized forms of *osteitis fibrosa*, the brown tumors in the bones, and the bone cysts in isolated parts of the skeleton.

(Dr. Jaffé, closing.) We have not particularly made up our minds about the localized *osteitis fibrosa*. However, a great number of conditions are really included under localized *osteitis fibrosa*, many of which are purely inflammatory lesions, which leave as a residue fibrous changes in the bone, because for some reason or other, the bone has not reconstructed itself normally. It appears to us quite possible that there may be one bone *osteitis fibrosa*, and at the present time we are inclined to view the lesion as due possibly to a lowered threshold, local in that bone, possibly on the basis of circulatory disturbances, which makes that bone peculiarly and particularly susceptible to the fibrosis-producing factors.

THE HISTIOGENESIS AND DEVELOPMENT OF RETICULUM; ITS WIDESPREAD OCCURRENCE IN THE ADULT ORGANISM. A New Method of Demonstration.  
James F. Rinehart (by invitation), San Francisco, Calif.

*Abstract.* A method of impregnating connective tissue substance, including reticulum and collagen, is given in which there is apparently a complete impregnation of such substances and in which adequate counterstaining is achieved.

inoculation being very similar to spontaneous leukemia and to Line I of Richter and McDowell. It would seem that if leukemic cells are introduced into the circulation, they are filtered out, and only after a certain degree of lymphoid hyperplasia has been attained do they invade the blood. Leukemia and aleukemic lymphadenosis are apparently essentially the same process, a conclusion that Richter and McDowell have likewise reached.

(Dr. Powsler, New York City.) It would seem that this leukemia is inheritable from one generation to the next. The offspring have a liability to leukemia.

(Dr. Richter). We first began systematic observations in the eighteenth generation of this line. It is now in the twenty-third generation.

(Dr. Powsler.) It is an inheritance due to some characteristic of some other organism, let us say white blood cells, or to some other condition which causes the organism to act that way; the point is that it is not a true genetic characteristic, but is inherited in the same way as syphilis, for example.

(Dr. Richter, closing.) In reply to Dr. Furth, the inoculation of other lines has been attempted, and in our experience has been negative so far. We have not attached very much importance to this because it has been recorded already that leukemia occurring in mice may be transmitted to other strains of mice. However, I feel that in the experiments of Furth the low percentage of "takes" may be explained by the fact that the mice into which the inoculations were made were not inbred, and therefore not uniform.

With regard to Dr. Powsler's question, we feel that the reappearance of leukemia in this strain is on a genetic basis, and we base this not only upon the appearance of leukemia in this strain, but on the ratios of susceptibility in hybrids which we have observed. Some of these observations were reported at the American Association for Cancer Research. It is not due to transmission through the mother, because hybrids with a leukemic male parent, and a non-leukemic female parent have also been susceptible. It is apparently not analogous to the so-called "inheritance" of syphilis or similar conditions.

EXPERIMENTAL OTITIS FIBROSA (FIBROUS OSTEODYSTROPHY) IN GUINEA PIGS ON NORMAL DIET, INJECTED WITH PARATHORMONE. H. L. Jaffé and (by invitation) A. Bodansky and J. E. Blair, New York City.

*Abstract.* It has been suspected for about twenty-five years that the parathyroids are in some way related to the bone dystrophies. Recently Mandl, and after him others, removed enlarged parathyroids in cases of otitis fibrosa cystica and reported rapid improvement of their patients, with cessation of the negative mineral balance.

No reports have appeared on the experimental production of the fibrous osteodystrophies by the use of parathyroid extract. Nor have bone changes that fulfill the requirements for the diagnosis of otitis fibrosa been produced experimentally by other means.

By subcutaneous injection of parathormone we have regularly produced in the guinea pig the generalized bone changes which satisfy all the criteria of otitis fibrosa, including the appearance of new bone (osteoid). The latter appears as soon as the reparative processes are permitted to operate with sufficient intensity. A guinea pig permitted to go for a few days without parathormone, after previous parathormone treatment, shows an abundance of osteoid tissue beneath the periosteum and endosteum, in the haversian canals, and in the fibrous tissue in the metaphysis just distal to the epiphyseal cartilage plate.

mitter, a rise in his temperature occurred, the height of temperature depending on the time he was in the field and his proximity to the apparatus. A rise of 2.2 degrees was caused in fifteen minutes and the response was very pronounced when a water-cooled 20 kilowatt radio tube was discharging from a six foot rod to ground with 60,000,000 alternations per second, of 15 kilovolts. Since diathermy with its relatively low rate of alternation of current is reputed to have valuable therapeutic applications due to the heat generated in the tissues, Dr. W. R. Whitney, director of the Research Laboratory of the General Electric Company, devised a modification of the radio transmitter which has become available for the study of experimental aseptic hyperthermia. The apparatus is a high frequency heater or oscillator on the principle of a short wave transmitter, except that the energy is concentrated between two plate electrodes instead of being directed from an aerial. Complete details of the machine are being published elsewhere. In the experiments I shall report, a wave length of 25 meters was used almost entirely, an amperage of 0.2 to 0.35, oscillating about 10,000,000 per second, a rate so rapid that no muscular contractions are produced.

Twenty-three dogs, twenty-one rats and four guinea pigs were exposed between the plate electrodes for variable periods of time. A temperature once reached could be maintained either by decreasing the voltage or by increasing the distance between the electrodes. A dog's temperature taken by rectum, or determined by thermocouple, could be raised as much as 7.1 degrees in thirty-seven minutes, 8.3 degrees in less than an hour, such rapid heating often causing death, but not always. Three dogs were kept at about 109° F from five to twelve hours. Twelve dogs were given from two to nine heatings that lasted from four hours to thirty hours, the temperatures varying from 107° to 112° F.

The white rat and the guinea pig could withstand about the same temperature as the dog and the tissue changes are practically the same for these three species under the same conditions of the experiment.

After removal from the oscillator, the temperature with few exceptions would return to normal in three hours. Repeated exposures would rarely give indication of loss of control of the heat-regulating center as shown by a prolonging of the pyretic state.

While being heated, the animal would become restless, salivate, pant, perspire, and the visible membranes become congested.

In some dogs placed too close to the electrodes, perspiration caused arcing, resulting in severe skin burns which healed very slowly. Rapid heating of rats often caused a disruption of the skin of the tail and severe burns at the base of the tail, with the skin finally sloughing off.

Postmortem examinations were performed as promptly as possible since post-mortem degeneration was very rapid in the highly heated tissues.

The animals that died or were killed while still at a high temperature showed the following conditions:

Acute congestion of all the organs. In addition, the heart showed focal hemorrhage, interstitial edema, fatty degeneration of muscle fibers. The lungs showed emphysema, atelectasis, small hemorrhages and increased secretion of bronchial mucus. Hemorrhages and necrosis of lymphoid tissue were present in the spleen; endothelial hyperplasia was noted. Often fibrin thrombi were present in the sinusoids. The gastro-intestinal tract showed a few mucosal hemorrhages with necrosis of lymphoid tissue, excess mucus secretion, mesenteric hemorrhage, and an acute inflammation of mucosa was often a coincident

The mesenchyme is shown to possess a fibrillar cytoplasm very closely related to reticulum and collagen. The differentiation of capillaries in loco in the mesenchyme is shown; the differentiation of mesenchyme into adult connective tissue is also indicated. Reticulum is shown to be a direct descendant of the mesenchyme, and evidence is presented of the probable transformation of reticulum into collagen. Reticulum fibrils are demonstrated as of universal occurrence in the capillary endothelium. Reticulum is identified with the basement membrane in the kidney, gastric mucosa and pancreas. The epithelioid cells of tuberculosis are shown to be capable of forming reticulum fibrils which may be transformed into collagen. By securing complete impregnation of this fiber substance and combining it with adequate counterstaining, a more complete concept of the capillary bed is achieved in which reticulum-lined spaces are shown to connect apparently isolated capillaries, as seen by ordinary staining methods.

**RAPID DIAGNOSIS OF INTRACRANIAL TUMORS BY SUPRAVITAL STUDY.** Louise Eisenhardt (by invitation) and Harvey Cushing, Boston, Mass.

*Abstract.* Description of a method of immediate diagnosis of intracranial tumors by supravital study of fresh smears is given. The method has been in use for the past two and one-half years and it is possible by this means to differentiate the various types of gliomas, for example, almost more easily than by the usual methods of fixed preparations.

#### *Discussion*

(Dr. W. C. MacCarty, Rochester, Minn.) I have been devoting the last twenty-five years of my life to the study of living tissues, and especially unfixed tissues, and should be among the first to congratulate Dr. Eisenhardt on this work. I was very much interested, for I visited Dr. Cushing's laboratory about a month ago and saw these slides in actual preparation. I can say also, in spite of the fact that these slides are very beautiful, that they do not compare with the originals from which the slides were made. I do not know just how many pathologists appreciate just what this service is to a surgeon, but those of us who have been intimately connected with surgical work know that he will act on the nature of the tumor. He must know the type of tumor; he cannot wait even for an hour, because an hour with a patient under anesthesia means something in brain surgery, so I hope you will take seriously this service which Dr. Eisenhardt has rendered, and apply the method. The method which she has shown is somewhat different from that which I have used in the last twenty-five years, but the end results are the same, and that is the important thing after all. This method gives much more beautiful results than any fixed method I know of.

(Dr. William Boyd, Winnipeg.) Can this method be used for autopsy material? Can it be used only on fresh, living material?

(Dr. Eisenhardt, closing.) These preparations are made only from fresh living material as soon as possible after it has been removed from the body. We have not attempted to use the method on autopsy material.

**AN EXPERIMENTAL STUDY OF THE EFFECTS OF HEAT INDUCED BY HIGH FREQUENCY ALTERNATING CURRENTS.** V. C. Jacobsen and (by invitation) K. Hosoi, Albany, N. Y.

*Abstract.* About two years ago it was noted in a laboratory of radio research that when a workman happened to get in the field of a short wave radio trans-

SPONTANEOUS AND EXPERIMENTAL SCHWANNOMAS. Pierre Masson and (by invitation) Charles Simard, Montreal, Can.

*Abstract.* Nageotte has shown that if a fragment of the sciatic nerve of the rabbit is excised from one side and grafted near the intact sciatic nerve of the other side, a voluminous tumor is produced by the sheaths of Schwann without participation of the axones — an artificial schwannoma. Comparative study of one of these tumors and of several spontaneous encapsulated neurinomas from man, shows a complete identity of their fundamental constituents. The structure, mode of collagen production, the manner of growth and development are the same in both types of tumor. A study of their characters shows that these constituents are different from connective tissue cells. In the building up of experimental schwannomas, connective tissue plays an important part, but in encapsulated spontaneous schwannomas the connective tissue plays only a minor part. The pure connective tissue aspect (fibromatous, myxomatous) of certain neurinomas corresponds to special morphological modification of Schwann cells which have been altered by subjection to certain circulatory conditions. The palisades that are found in many spontaneous schwannomas are derived from the sheath of Schwann and are perhaps characteristic of the type of tumor derived from sensory nerves.

QUANTITATIVE OBSERVATIONS ON THE SEMILUNAR VALVES OF THE HEART. Paul Gross (by invitation), Cleveland, O.

*Abstract.* A method for the determination of the surface area of valves is described. The surface area of the cusps of a semilunar valve with a large ring is commensurately greater than that of a valve with a normal ring. Valves with normal and large rings present no significant differences in the height of the cusps. Elongation of the valve rings of hearts at autopsy is associated with a decrease in the height of the cusps. It is probable, therefore, that as a valve ring increases in size the cusps increase in height. The surface area of the semilunar valves is 40 to 70 per cent in excess of a calculated minimal closing surface. The percentage of excess has no correlation with the size of the valve ring. A semilunar valve is incompetent if the average height of the cusps is equal to or less than the radius of the valve ring.

STUDIES ON THE PATHOGENESIS OF BACTERIAL ENDOCARDITIS. Robert Koch and Kurt Semsroth (by invitation), Pittsburgh, Pa.

*Abstract.* Injections of casein or killed streptococci, a prerequisite for the development of bacterial endocarditis in our experiments, lead neither to degenerative changes of the endocardium nor to a transformation of the endothelial coat of the endocardium into phagocytes. Experimental studies on the vital staining of the cells of the reticulo-endothelial system, however, reveal that subsequent to injections of casein or killed streptococci the reticulo-endothelial system loses to a considerable extent the ability to develop granular deposits of dyes, if the dyes are injected in the form of *solutions*, while the phagocytosis of *colloidal* particles of the same dyes is not disturbed.

Experiments were carried out to determine whether or not the functional disturbance of the reticulo-endothelial system, reported above, influences the degree of the deleterious effect of toxic bacterial substances on the heart. Control rabbits were given doses of a diphtheria toxin or typhoid vaccine which did not lead to inflammatory reactions of the endocardium nor to a myocarditis. Analo-

infection. Cloudy swelling, fatty degeneration, focal necrosis, focal hemorrhage, and depletion of glycogen were present in the liver. The pancreas was normal. The kidneys showed cloudy swelling, hydrops, fatty degeneration in Henle's and convoluted tubules. Focal hemorrhage, increased visible fat, hemorrhage in the capsular fat were seen in the adrenals. Dogs' testes were usually normal. Rats' testes showed much exfoliation of germinal epithelium and proliferation of Sertoli cells.

One female bulldog heated to 108° F for nineteen hours had a breast carcinoma which appeared unaffected.

The striated muscle showed glycogen depletion. The bone marrow was hyperactive. The thyroid was normal. Congestion, focal hemorrhage and chromatolysis of ganglion cells was noted in the brain. Chromatolysis of ganglion cells was present in the spinal cord.

Dogs and rats heated many times and the temperatures allowed to return to normal showed fewer changes in their organs. Congestion was regularly present but cloudy swelling and fatty degeneration were less frequently seen. Loss of weight was pronounced and the fat depots showed loss of fat. The weight was usually recovered rapidly, however, by the drinking of water. The pulmonary emphysema in the animals given one heating was probably due in part to an acidosis of lactic acid nature and not to ketosis.

The heat induced by this method is an internal heat, probably caused as Hosmer suggests, by an increased vibration of molecules of the cells produced by their alternate attraction to each of the electrode plates in turn.

From a study of the tissues of these experimental animals, it is seen that there is little to suggest any fundamental difference between the heat induced by this oscillating current and the hyperthermia in febrile disease, or that produced by external applications. Concomitant pulmonary or gastro-intestinal infection was present in slight degree in only a few animals. The dangers of the method are not entirely known. The cutaneous burns were preventable. Schereschewsky found experimental mouse cancer unfavorably influenced by this form of heating. Its application to various chronic diseases such as arthritis and paresis seems logical and such work is under way.

The pathology of fever can be studied more thoroughly than ever before by this method and yet its clinical application must be preceded by much fundamental work, since acidosis, and later alkalosis and tetany, cloudy swelling and fatty degeneration of parenchymatous organs, and deleterious effects upon the germinal epithelium of testes and ovaries are definite pathological states to be avoided in therapy if possible.

Finally, it seems quite in order in this anniversary year of Dr. William H. Welch to say that his Cartwright lectures given in New York in 1888 still constitute our greatest source of information concerning the pathology and etiology of fever, little of note having since been added.

#### *Discussion*

(Dr. Robert Koch, Pittsburgh.) At the Western Pennsylvania Hospital in Pittsburgh, Drs. Szymanowski and Hicks are experimenting on the biological effect of short wave radiation. The experiments show very clearly that radiation of this kind produces other effects besides the heat effect. In complicated systems like the animal body, the heat effect is so pronounced that other effects are masked. Simple systems like colloidal solutions or diphtheria toxin offer a better means for studying all the biological effects of short wave rays.

My second point refers to agglutination, complement fixation, and other serological reactions. Many investigators have employed bacteria grown on media containing serum. Dr. Cecil, in the studies reported to-day, has avoided this error.

It was reported by Olitsky and Bernstein in 1916, and by Olitsky and Denzer in 1917 that rabbits injected with bacteria grown on media containing serum responded by producing antibody against the organisms and against the serum proteins. Such immune sera are capable of giving non-specific reactions with various other bacteria cultivated upon the same kind of serum. Even after the fourth washing (saline solution) yields no evidence of serum proteins, the same result may ensue. These studies have been confirmed by F. S. Jones. In a paper published last year by Hooker and Anderson in the *Journal of Immunology* (a preliminary report of which appeared in 1924) this subject is well emphasized. In the course of a very elaborate and careful investigation of 616 alpha streptococci, cross-agglutination reactions were found in less than 3 per cent. The non-hemolytic streptococci are very individualistic in their characteristics, and undergo changes rather easily. I would not lay stress, for example, on the slight differences found by Dr. Cecil and his co-workers in the streptococci obtained in rheumatic fever, and those found in the cases of infectious arthritis.

What is the present status of the question of the etiological relationship of streptococci to rheumatic fever?

There are facts that speak for and against it. The final proof is not yet forthcoming. We know that similar streptococci are found in cases of subacute bacterial endocarditis and in the occasional instances of mixed infections (both active) of the two conditions. We know that they may be found in the blood without endocarditis appearing, in severe or mild local infections. What is most important is the fact that they may be found as transitory invaders in the greatest variety of febrile and non-febrile disease. In the last two cases of Hodgkin's disease that I have observed, non-hemolytic streptococci were present in the blood. In the second case, three positive cultures were obtained by Dr. Goldzieher (to whom I am indebted for permission to cite this case to you). In lymph nodes from a number of diseases, non-hemolytic streptococci have also been found in a viable condition. The studies of E. Z. Epstein and M. A. Kugel, that non-hemolytic streptococci may be cultivated in nearly all cadavers from the spleen and bone marrow, and in the heart muscle as well as valves that show no endocarditis; in as high a figure as (approximately) 50 per cent are of great significance.

It would have been of advantage if febrile cases had been employed as controls in the studies just reported. The fact, however, that the investigations revealed so few positive results in the fever-free control cases, except for those of chronic infectious arthritis in which they were so numerous, compels our attention.

(Dr. B. J. Clawson, Minneapolis). It seems to me this is pretty nearly the last link in the chain in confirming the belief that streptococci are the causal agent in acute rheumatic fever. The fact that these organisms are found in the joints is important; if they were found only in the blood, they might be secondary invaders, but when we have them present in the joints in such a high percentage of cases, it is pretty hard to think we have a secondary invader. It is also true of the organisms in the blood; those of you who have attempted to culture the blood of patients who are not as ill as those with acute rheumatic fever, find it

gous doses were given to rabbits that had received previously a series of injections of casein or streptococci vaccine. These animals developed a focal interstitial myocarditis. Furthermore, proliferative and exudative inflammatory reactions of the endocardium with marked degenerative changes were observed. It is inferred that the functional disturbance of the reticulo-endothelium system reported in the preceding paper is associated with an increased vulnerability of the heart to the action of bacterial products.

CHARACTERISTICS OF STREPTOCOCCI ISOLATED FROM PATIENTS WITH RHEUMATIC FEVER AND CHRONIC INFECTIOUS ARTHRITIS. R. L. Cecil and (by invitation) E. E. Nicholls and W. J. Stainsby, New York City.

*Abstract.* During the past three years, streptococci have been isolated frequently from the blood and joints of patients with acute rheumatic fever and chronic infectious arthritis. The technique consists in taking large quantities of blood and observing the cultures over a long period of time.

83.3 per cent of the strains isolated from cases of chronic infectious arthritis appear to belong to one biological group. These strains have been designated by us as "typical strains." They seem to fall into the alpha prime group of streptococci.

The streptococci recovered from cases of acute rheumatic fever belong to the *Streptococcus viridans* group although the methemoglobin formation is frequently difficult to demonstrate. Cross-agglutination tests show that some of these organisms tend to fall into biological groups.

Agglutination reactions with the serums of patients with chronic infectious arthritis against the antigen of any of the typical strains are very striking. The serums agglutinate to a very high titer, as high as 1:5120 or more. This condition is not found with control serums. Serums of patients with acute rheumatic fever agglutinate some of the rheumatic fever strains to a higher titer than do serums from control cases.

### Discussion

(Dr. Emanuel Libman, New York City.) Dr. Cecil requested me to discuss this presentation. He asked me to state whether or not I was of the opinion that these streptococci came from the air or were in the culture media. I have no doubt that they came from the blood of the patients.

I will not go deeply into the controversial side of the subject under discussion. My main purpose is to draw attention to the tremendous amount of time and energy that often has been lost in connection with the bacteriological study of streptococci in general — and the non-hemolytic streptococci in particular.

In the first place, most of the investigations of the fermentation reactions of these organisms are of little value as the more favorable media have not been employed. Many studies have been performed with agar media without the addition of serum. In the course of elaborate investigations made many years ago, we found that this medium is the poorest for the purpose. The next best is a bouillon medium without serum. Agar plus serum is much better and bouillon plus serum is still more favorable. Even the last-mentioned is not an optimum medium. The Hiss serum-water medium is of little value unless peptone is added (as first suggested by Buerger). Even then it is not nearly as good as the bouillon-serum method. For over twenty years I have been convinced that we must be careful of classifying non-hemolytic streptococci by means of their fermentative power on various carbohydrates.



Aschoff body, will be found at an earlier stage of the lesion equally as well, and if they will persist through the later stages of the same lesion.

(Dr. Emanuel Libman, New York City.) I would like to clarify one point. I do not believe that Dr. Gross meant to say that Fahr described only one case. Did he not describe a small endemic? The findings were exceptional, and therefore there is a strong possibility that he happened to be dealing with a group of cases in which scarlet fever and rheumatic fever coexisted.

(Dr. H. F. Swift, New York City.) I would like to point out in connection with what Dr. Libman has said that Siegmund only recently has reported a large series of cases where death occurred several weeks following the acute stages of scarlet fever, and stated that as the time lengthened between the onset of scarlet fever and the time of death, the final lesions of the cardiovascular system approximated more and more those seen in rheumatic fever; hence the time element appears to be an important factor. As far as I can recall, all of Fahr's cases were fatal soon after the onset of scarlet fever; and when considered in connection with the observations of Siegmund, bring up a most important question in the general consideration of this problem — the time between the onset of the scarlet fever and the occurrence of the peculiar type of lesion. Only after the noxious agent has been active for several weeks or months does the tissue appear to respond in the peculiar proliferative manner observed in either true rheumatic fever or scarlatinal rheumatism and carditis.

(Dr. Herman Schwarz, New York City.) In a case of scarlet fever with joint manifestations, I had the opportunity of examining the heart seven weeks after the onset of the disease. There was a definite double murmur at the apex, but what was more important, I found typical rheumatic nodules on both olecranon processes. It might be concluded that in this case the so-called scarlatinal rheumatism was genuine rheumatism.

(Dr. Gross, closing.) In answer to Dr. Clawson's request for a definition of the typical Aschoff body, may I say I had hoped our demonstration would serve this purpose. However, if Dr. Clawson will tell me of what stage in the cycle he would like to have a definition of the typical Aschoff body, I shall be glad to give it to him. I have tried to point out the fact that the Aschoff body is not a static structure. It is fluid and continually changing. At one characteristic stage, after the syncytium has partly broken down, the cell protoplasm is generally supple, voluminous and basophilic; the cell edges are ragged; there are small masses of naked protoplasm about; there are also masses of swollen collagen and a peculiar "chicken wire" silver-staining reticulum to be seen; the nuclei are vesicular, relatively clear, with a dense perinuclear membrane and a conspicuous dark central nucleolus (owl-eyed). Giant cells may or may not be present. There is no elastica and rarely fibrin to be found. We have not found this picture thus far in any other condition. As I said before, however, my definition will have to vary with the stage of the cycle described.

Like Dr. Clawson, we were also puzzled as to how certain we could be about which was the tail and which the beginning of the cycle. When one tries to work out the life cycle of a lesion occurring in the human, where one cannot have experimental control, there is always an element of uncertainty. But there are certain factors which one can use as a guide, for example, the clinical history, the appearance of fresh foci of polymorphonuclear leucocytes, the appearance of fresh verrucae, the maturity of the verrucae and, best of all, the state of swelling of the collagen and the maturity of the connective tissue. In working out the

quite unusual to demonstrate streptococci in the blood. It is true with Hodgkin's disease, and late in carcinoma it is a common thing to find streptococci in the blood. But with people who are not very ill it is quite unusual to find streptococci in the blood. I have followed Dr. Cecil's work very carefully, and I should like to ask if he has come to any conclusion as to why the organism grows so slowly at first, and then after you get it out on proper culture media, it grows so easily.

(Dr. V. H. Moon, Philadelphia.) I am greatly interested in this work of Cecil and his associates, and while the subject of the etiology of acute rheumatic fever is under discussion, I should like to remind those who were not here yesterday of some results we have obtained by the inoculation of animals with *Streptococcus viridans* obtained from blood culture and from the heart valves of a case diagnosed clinically as bacterial endocarditis. The inoculation resulted in the formation of the verrucous type of endocarditis on the mitral valve and in the auricle, the formation of nodules in the walls of the blood vessels, in the heart muscle, and in skeletal muscles, and of fibrous nodules histologically identical with the subcutaneous nodules in acute rheumatic fever. I think this should be borne in mind as confirmatory of the evidence of the relationship of streptococci to acute rheumatic fever.

(Dr. Nichols, closing.) In answer to Dr. Clawson's question, we have not found any explanation for the slow growth of the organisms.

HISTOLOGICAL STUDIES ON THE ASCHOFF BODY. Louis Gross and (by invitation) Joseph C. Ehrlich, New York City.

*Abstract.* The descriptions of Aschoff bodies found in the literature are in many cases confusing. There are many disagreements on the histological appearance of these bodies, mainly because various authors depict different structures as well as different phases in the evolution of the same structure. This also accounts to a certain extent for differences of opinion on the origin of the cells found in Aschoff bodies. By using various histological methods on a large amount of autopsy material, a series of pictures have been found which suggest a fairly definite life cycle for the Aschoff body. In certain cases, at least, the Aschoff body takes the form of a well developed syncytium surrounding swollen collagen as one characteristic phase in the life cycle. Several phases of this cycle appear to be pathognomic of rheumatism. It is believed that if this cycle is thoroughly understood there will be less difficulty in recognizing these structures. The Aschoff body is shown to possess a characteristic reticulum which also appears to go through a recognizable life cycle.

#### Discussion

(Dr. B. J. Clawson, Minneapolis.) I want to repeat the same question I asked last year, and ask Dr. Gross to give a definition of a typical Aschoff nodule. Another question I should like to ask is how he knows that one end or another is the beginning of the life cycle of an Aschoff nodule. He certainly demonstrated that the Aschoff nodule may take many forms. There may be various stages. Another point about those elongated ones, I should like to know what would happen if they were cut the other way, that is, transversely rather than longitudinally.

(Dr. V. H. Moon, Philadelphia.) I should like to ask whether the eight or nine characteristics which Dr. Gross states must be present for the diagnosis of an

*Discussion*

(Dr. H. T. Karsner, Cleveland.) The tissue response described by Dr. Clawson in these experiments suggests the possibility that he may be dealing with anaphylactic inflammation as described by Opie. Will Dr. Clawson state whether or not the bacteria were washed for use in sensitization, immunization or subsequent injection?

(Dr. Homer Swift, New York City.) Dr. Clawson's report is more or less in confirmation of what we suggested last year. There is one point well worth emphasizing, namely, the qualitative relationship, the type of response and the type of soil in which the bacterium is planted. Dr. Karsner has brought up a point which also appears well worth keeping in mind, namely, the relationship of bacterial hypersensitiveness to that phenomenon which Opie called anaphylactic inflammation, to which Rössle gave the name of hyperergic inflammation, where the argument is based upon experiments done with a different type of antigen. If one uses for preliminary sensitization a coagulable protein such as egg-white, he obtains after reinoculation into the tissues a hyperergic or an anaphylactic type of inflammation that is increased in intensity more or less parallel with the increase in the concentration of circulating immune bodies. With whole bacterial antigens, that does not hold, as is brought out by Dr. Clawson's work. The hyperergic inflammation following injection of bacteria into the tissues is determined by the preceding mode of inoculation. If it has been into the subcutaneous tissue, and now I am dealing with streptococci, the animal's reactivity is increased, so that a so-called hyperergic type of inflammation results from reinoculation. If the dose of antigen be accurately titrated, and the lesions resulting from various doses be excised daily and compared, not only a histiocytic response will be observed in the hyperergic inflammation, but various types of exudative reaction will be found early. On the other hand, an animal with high antibody content in its serum resulting from previous intravenous inoculation will respond to intracutaneous injection of small or medium-sized doses only with a histiocytic mesenchymal reaction; in the first the response may well be termed hyperergic inflammation, and in the second immune hypo-ergic reaction.

(Dr. Louis Gros, New York City.) In a personal conversation with Dr. Clawson he very kindly informed me of the fact that he had obtained similar subcutaneous nodules in the skin of rabbits injected with hay bacilli. I should like to know whether he was able to discern any histological differences between the nodules produced by the hay bacilli and those produced by the streptococci.

(Dr. Clawson, closing.) I have avoided the term anaphylactic on purpose, for the reason that Dr. Swift brought out. There is a disagreement among immunologists as to just what the hypersensitive reaction, such as the tuberculin reaction, is, and realizing that, I used the term hypersensitiveness. If the hypersensitiveness produced by bacterial proteins is anaphylaxis, then I think this is an anaphylactic reaction. I am not certain it is. It is not the same thing which we get by injecting horse serum. This reaction seems to depend on something in those abscesses. What this is, I do not know, and I am waiting for some immunologist to tell us.

In reply to Dr. Karsner's question about washing the organisms, the first were not washed. I used a broth culture of organisms mixed with agar. The standardized organisms were washed.

The quantitative reaction should be emphasized, and while these injections

life cycle of the Aschoff body we had to be guided not only by such factors as these, but also by how well these factors dovetailed into each other.

With regard to the appearance of a polarized Aschoff body when looked at on end, we have sectioned the Aschoff bodies serially, and at the polarized stage the end view shows a central nucleus surrounded by very scanty protoplasm. The hyaline connective tissue is replaced largely by delicate fibrillae.

Dr. Moon has asked whether these various characteristics persist. Let me answer this question categorically to a certain extent. The protoplasm basophilia appears just before the height of the cycle and goes through to the end. The reticulum starts at the beginning and persists to the end stage, that is, the stage of mature connective tissue when the silver-staining reticulum completely disappears. The nuclei are characteristic at the height of the cycle and continue almost to the very last stages.

With regard to Dr. Swift's remark about the element of time, I agree with him thoroughly of course. We would be illogical if we denied the possible rôle which time may play in this connection, let alone the question as to whether rheumatism is a modification of scarlet fever. It seems to me, however, that at the present time one has no right to assume otherwise than that this disease, for some unknown reason, seems at times to follow on scarlet fever. One would have to have other evidence to link the two diseases under one etiology.

EXPERIMENTAL STREPTOCOCCIC INFLAMMATION IN IMMUNE AND HYPERSENSITIVE ANIMALS WITH SPECIAL REFERENCE TO THE PATHOGENESIS OF RHEUMATIC LESIONS. Benjamin J. Clawson, Minneapolis, Minn.

*Abstract.* The experiments were performed with the following three groups of animals: (1) animals which had not been injected (normal animals); (2) animals which had been injected intravenously with streptococci (immune animals); (3) animals which had been injected subcutaneously in one area with agar at 45° C, heavily seeded with streptococci (allergic or hypersensitive animals). Each animal was injected in ten places in the subcutaneous tissues on the right side with a 1:100 suspension of streptococci and in ten places on the left side with a 1:1000 dilution of the suspension.

The polyblastic type of microscopic cellular reaction was found in all three groups. No difference could be detected in the character of the reaction in the nodules. It appears evident that the polyblastic type of reaction, which is characteristic of the lesions found in human rheumatic cases, does not depend primarily upon a hypersensitive stage when produced experimentally in animals. If doses sufficiently large are given, this reaction may be produced in both normal and immune animals, as well as in the hypersensitive animals.

The relationship between allergy and the polyblastic type of reaction appears to be a quantitative one. This quantitative relationship may help to explain the pathogenesis of human rheumatic lesions in many cases. Since streptococci have not been found in large numbers in the blood and joints of patients having acute rheumatic fever, it has been difficult to understand the extensive lesions; but when it is taken into account that small doses of streptococci will produce extensive reactions in hypersensitive animals and that high percentages of patients having acute rheumatic fever are hypersensitive to streptococci, the pathogenesis of the human lesions can be understood more readily.

tions of the nodules of chronic rheumatoid arthritis, I was struck by the extreme analogy of these degenerative changes with those which we observed in cases of viridans-endocarditis associated with Aschoff bodies. Furthermore, that particular type of gelatinous necrosis of the connective tissue is frequently seen in the vascular lesions in chronic streptococcus sepsis, all of which might lead one to stress the probability of the causative relation of streptococci to these lesions. On the other hand, I do not believe that such a conclusion can be made, since Klinge of Leipzig recently has noticed that very type of gelatinous necrosis in the connective tissue of rabbits hypersensitive to horse serum, and which were subsequently injected into the knee joints with horse serum. He claims to have induced lesions in the synovia, in the periarticular tissue, in the connective tissue of the arteries removed from the site of injection, in the myocardium and endocardium, characterized by the same type of gelatinous necrosis and by proliferation of large mononuclear elements. In the light of these results it appears that a certain state of the host is one determining factor in the pathogenesis of rheumatic lesions. Another, but not the only, factor may in man well be the invasion of the host by streptococci.

(Dr. Dawson, closing.) We have had no difficulty in differentiating any of these cases from cases of gout. We have studied some nodules from gouty cases, and their structure is apparently entirely different.

A NEW MENINGOCOCCUS-LIKE ORGANISM (*NEISSERIA FLAVESCENS*, N. SP.)  
FROM EPIDEMIC MENINGITIS. Sara E. Branham (by invitation), Washington, D. C.

*Abstract.* Of 155 strains of meningococci isolated from cases of epidemic cerebrospinal meningitis in the United States during 1928 and 1929, fourteen strains were uniformly atypical. These were among fifty spinal fluid strains received from one city during a single outbreak, and thirty-six of which, or 72 per cent, fell into the four antigenic groups of Gordon's classification. The remaining fourteen, or 28 per cent, formed a homogeneous serological group. There was no cross-agglutination with any of the four usual groups of meningococci; neither was any of these strains agglutinated by polyvalent commercial antimeningococcus sera.

Aside from this serological difference these fourteen uniform strains varied from the typical meningococci in pigment production and in total lack of fermentative ability. They did not correspond with any of the gram-negative cocci previously described.

The cases with which these cases were associated were subacute and ran a relatively long course. Eleven of the patients were given polyvalent antimeningococcus serum freely, and seven made a complete recovery.

This homogeneous group of fourteen strains does not conform to the usual conception of the meningococcus, although the strains were isolated from the spinal fluid of meningitis cases during an epidemic in which all four of the usual serological types of meningococci were found. To call them meningococci would alter the definition of a meningococcus and plunge the classifications of the meningococcus group into confusion. For the present we shall refer to this new group of fourteen strains as *Neisseria flavescens*, n. sp.

The fact that 28 per cent of the strains isolated in one locality, or more than 9 per cent of those received from the country at large, are members of this restricted group suggests the importance of giving special attention to it.

were standardized fairly closely, we are now carrying on a more accurate method of standardization. In all these cases, control animals were run with the same doses of organisms each time.

In reply to the question about the hay bacillus, this reaction is not specific for streptococci. We have used the hay bacillus in the same way, and have been able to produce the same reaction. In fact, we have been able to reproduce with the hay bacillus the secondary reaction reported by Swift and his co-workers.

**SUBCUTANEOUS NODULES IN CHRONIC INFECTIOUS ARTHRITIS.** M. H. Dawson (by invitation) and A. M. Pappenheimer, New York City.

*Abstract.* The occurrence of subcutaneous nodules in patients suffering from rheumatic fever has long been recognized and has been the subject of intensive investigation by many workers. Less attention, however, has been paid to the subcutaneous nodules that occur in patients afflicted with rheumatoid (chronic infectious) arthritis.

During the past year in the Arthritic Clinic of the Presbyterian Hospital, subcutaneous nodules have been observed in twenty-three patients. All of the patients presented the typical clinical picture of rheumatoid or chronic infectious arthritis. Nodules were excised from eleven of these patients and subjected to careful histological and bacteriological examination.

All the material examined has shown a striking, uniform and characteristic picture. The essential histological features may be summarized as follows:

(1) an area of central necrosis, apparently due in its earliest stages to a gelatinous swelling and degeneration of individual collagen bundles; (2) a surrounding zone of peculiar and characteristically arranged large mononuclear cells; (3) an enclosing zone of dense and relatively avascular fibrous tissue.

The blood vessels in the area of the nodule itself rarely show significant changes, but the arterioles and capillaries in the surrounding tissue are the site of characteristic lesions.

There is a striking resemblance between the histological appearance of these nodules and those which occur in rheumatic fever.

The bacteriological investigations have been entirely negative.

### *Discussion*

(Dr. Emanuel Libman, New York City.) I want to speak because I feel that this valuable contribution, so excellently presented, should not be passed without any discussion. Were any nodules found near the sacro-iliac synchondroses? I ask this question because I have been much interested in the nodules found in that situation by Goldscheider who believes that they are of gouty origin. A thorough study of them has not been made. I feel that they indicate a metabolic disturbance, and that it is still to be proved that they indicate real gout. Goldscheider has found urate crystals in some of them, and believes that when such crystals are not found, they had been present and had been absorbed. It would be interesting to determine whether or not all nodules over the sacro-iliac synchondroses are of metabolic origin. Possibly some may be found to be of infectious origin, or even of combined metabolic and infectious origin.

(Dr. Kurt Semsroth, Pittsburgh.) I was particularly interested in the fact that Dr. Dawson referred to the type of necrosis as gelatinous. If I was not mistaken, the particular type of necrosis, starting with the gelatinous swelling of the connective tissue, has been stressed by Dr. Dawson. When I examined his sec-

- SARCOMA IN WILD MALLARD DUCKS. David L. Belding, Boston.
- THE NEUTROTROPISM IN THE HUMAN TESTICLE AND HYPOPHYSIS. Louis Berger (by invitation), Quebec, Can.
- A NEW MENINGOCOCCUS-LIKE ORGANISM (*NEISSERIA FLAVESCENS*, N. SP.) FROM EPIDEMIC MENINGITIS. Sara E. Branham (by invitation), Washington, D. C. (*cf. Abstracts*).
- REPORT OF CASE OF ACUTE LEUKEMIA WITH AUTOPSY. Baxter L. Crawford, Philadelphia, Pa.
- EXPERIMENTAL PNEUMOCOCCUS INFECTION IN THE HORSE. Theodore Curphey (by invitation), New York City.
- THE BEHAVIOR OF THE COLON BACILLUS AND ITS SPECIFIC BACTERIOPHAGE IN URINE CULTURES. Frances C. Frisbee (by invitation) and W. J. MacNeal, New York City.
- ERYTHROLEUCOSIS AND THE ANEMIAS OF THE FOWL. J. Furth (by invitation), Philadelphia, Pa.
- METASTASIZING CARCINOID TUMOR OF JEJUNUM. Istvan Gaspar (by invitation), Rochester, N. Y.
- STRUMA OVARII. J. L. Goforth, Dallas, Texas.
- A FATAL CASE OF GENERALIZED MONILIASIS WITH SPECIAL REFERENCE TO THE PATHOLOGY. S. R. Haythorn and (by invitation) G. H. Robinson and L. W. Johnson, Pittsburgh, Pa.
- ATTEMPTED CHEMOTHERAPY IN EXPERIMENTAL RABIES. A. Hoyt (by invitation) and C. W. Jungeblut, New York City (*cf. Abstracts*).
- SMALL CELL CANCERS OF THE LUNG. Howard T. Karsner and Otto Saphir, Cleveland, O.
- NEOPLASMA OF THE PLEURA — MESOTHELIOMA AND FIBROSARCOMA. Paul Klemperer and (by invitation) C. B. Rabin, New York City.
- STANDARDIZATION OF ANTIGEN. B. S. Levine (by invitation), Hines, Ill.
- BLOOD CHANGES DURING TRYPANOSOME SEPTICEMIA. Richard W. Linton (by invitation), New York City.
- RELATION OF THE FOLLICLES AND THE FOLLICULAR ARTERIES OF THE SPLEEN. W. J. MacNeal and (by invitation) J. M. Ravid, New York City.
- STUDIES IN BARTONELLA MURIS ANEMIA. J. Marmorston-Gottesman (by invitation) and David Perla, New York City.
- THE EVOLUTION OF MASSIVE PULMONARY TUBERCULOSIS IN THE RABBIT. E. M. Medlar and (by invitation) K. T. Sasano, Mt. McGregor, N. Y.
- THE RELATION OF THE EPITHELIUM TO THE MUCOSA IN PACHYDERMIA LARYNGIS. Louise H. Meeker, New York City.
- FIXATION OF IRON BY AN INFLAMMATORY REACTION. Valy Menkin (by invitation), Philadelphia, Pa.
- THE TOTAL NUMBER OF GLOMERULI IN THE NORMAL HUMAN KIDNEY. Robert A. Moore, Cleveland, O.
- FIBROSIS OF LUNG CONSEQUENT TO PULMONARY ARTERIOCAPILLARY FIBROSIS. Eli Moschowitz, New York City.

ATTEMPTED CHEMOTHERAPY IN EXPERIMENTAL RABIES. A. Hoyt (by invitation) and C. W. Jungeblut, New York City.

*Abstract.* A typical and constant infection was produced in white mice by the intracerebral injection of fixed virus. The animals usually showed beginning paralysis in about six days, and died on the eighth or ninth day.

In experiments involving 255 mice, which were injected intracerebrally with 0.02 cc. of different dilutions of virus, the approximate minimum fatal dose was 1:320 dilution of this virus. Though the majority of animals injected with amounts of virus up to as little as 1:1280 died of rabies, a few survived. Beyond this point the percentage of survivals increased rapidly.

Attempts were made to protect mice against rabic infection produced by intracerebral injection of one or more minimum lethal doses of fixed virus, by prophylactic treatment with silversalvarsan, neosalvarsan, tryparsamide, Bayer 205 (Germanin), plasmochin, optochin and quinine bisulphate. The drugs, with the exception of quinine bisulphate, were injected intravenously in doses as large as could be tolerated. Treatment was ordinarily started on the day of the infection; in some cases it was continued by one or more subsequent doses of the drug.

None of the drugs used displayed the slightest prophylactic or therapeutic effect on the infection, under the conditions of the experiment. There was, however, often a slight prolongation of the incubation period of the disease in some of the treated mice, as compared with that of the controls. This was especially noticeable with silversalvarsan and Bayer 205 (Germanin), while the incubation period appeared to be actually shortened by the administration of the quinine compounds. Caution should be used in interpreting the above results, as the incubation period of a few mice, treated with distilled water only, was likewise slightly lengthened.

BLOOD CHANGES DURING TRYPANOSOME SEPTICEMIA. Richard W. Linton (by invitation), New York City.

*Abstract.* A study of some aspects of the blood chemistry of rats with acute septicemia due to infection with *Trypanosoma equiperdum*, yielded the following results: The carbon dioxide capacity of the serum was markedly lowered early in the disease. The non-protein nitrogen and uric acid constituents in the blood were increased in the terminal stages. The kidneys also showed terminal degenerative changes. The cholesterol remained unchanged throughout. Lecithin was markedly increased, most of the observations showing a 20 to 50 per cent rise in this substance. Liver glycogen was lower than normal in the early stages and could not be demonstrated in the later stages of the infection. The blood sugar remained normal until a very late period in the disease.

#### READ BY TITLE

COR BIATRIATUM TRILOCULARE, COMPLICATED BY OTHER GRAVE CARDIAC ANOMALIES AND COMPENSATORY DEVELOPMENT OF ANASTOMOSES BETWEEN BRONCHIAL AND PULMONARY CIRCULATIONS WITH FORMATION OF CONGENITAL ARTERIOVENOUS ANEURYSM IN LUNGS. FROM A MAN AGED 20 YEARS WITH COMPLETE CONGENITAL HEART-BLOCK AND DEATH FROM PULMONARY HEMORRHAGE. Maude E. Abbott, Montreal, Can.

MUCOID NEOPLASMS OF THE URINARY TRACT. Nicholas M. Alter (by invitation) and Joseph M. McCarthy (by invitation), New York City.





CASE OF MYELOMA WITH UNUSUAL AMYLOID DEPOSITION. Beryl H. Paige, New York City.

THE BIOLOGY OF INFLAMMATION. Ernest Pribram, Chicago, Ill.

ENDOCARDIAL POCKETS OF THE LEFT AURICLE AND VENTRICLE. Otto Saphir, Chicago, Ill.

FULMINANT GENERALIZED INFECTION WITH THE PFEIFFER BACILLUS. Adele E. Sheplar and Lawrence Sophian (by invitation), and W. J. MacNeal, New York City.

GLOMERIC TUMOR OF THE SKIN. Charles Simard (by invitation), Montreal, Can.

## RETICULIN OF THE AREOLAR TISSUE

With a hypodermic syringe, 1 or 2 cc. of Locke's solution are injected into the subcutaneous areolar tissue of the abdomen of a rat. There is thus produced a well defined, edematous tumor in which the connective tissue elements, normally compressed so closely that it is impossible to study them, are separated uniformly so that their arrangement can be examined in all of its details.<sup>4</sup> This valuable technique is due to Ranvier.

The edematous tumor looks like a transparent jelly. A thin slice cut with the curved scissors and immersed in Locke's solution holds its shape. It may be mounted between slide and cover glass and examined with the ultramicroscope, which reveals perfectly the collagen bundles and the reticulin of which this tissue consists.<sup>5</sup> The entire thickness of the abdominal wall may be cut out around the tumor, fixed in Zenker's or Helly's fluid and embedded in paraffin, in order to obtain thin sections for staining with Mallory's method; or it may be embedded in celloidin, thick sections impregnated with silver by the usual methods and mounted in balsam, after having dissolved and removed the celloidin.

The latter preparations are particularly instructive (Fig. 1). Despite their thickness, which may exceed 50 microns, they are perfectly transparent. Changing the focus enables us to follow the fibers over long stretches. The coarse collagen bundles are ribbon-like, wavy along their borders as along their surfaces and are pale yellow. The opaque black reticulin extends out into the interstices so that all of its fibrils and meshes are seen distinctly. Some parts form networks of two dimensions which, at their borders, are continuous with networks of three dimensions. In all of these networks the elements are uniformly separated by the injected fluid so that the relations between them are not altered; nowhere is there the least tearing of tissue. The difference from the normal state is of the same order as the difference between lace which is wet and crumpled and the same lace spread out, each thread being detached on an optically empty ground. Moreover, if the edematous tumor is left to follow its natural course in the living animal, it will disappear by itself in a short time and there is nothing, either to the naked eye or on microscopic examination, to reveal its former location; no lesion remains.

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## RETICULIN \*

J. NAGEOTTE AND L. GUYON

*(From the Laboratory of Comparative Histology, Collège de France, Paris, France.)*

Since the fundamental work of Mall,<sup>1</sup> histologists have busied themselves much with reticulin. Interest in this form of connective tissue increased when silver impregnation provided the means for more precise morphological study. One fact particularly attracted attention; in successful preparations, reticulin stains black while collagen bundles stain yellow, thereby fortifying Mall's conclusion that reticulin, yielding no gelatin on boiling, is a substance different from collagen. Many authors now designate it as the argyrophil reticulum, contrasting it with the collagen bundles, which are not believed to be arranged in a network.

Partisans of the unitary hypothesis, Mallory and Parker<sup>2</sup> among them, insist that reticulin does not differ essentially from the collagenous framework with which its substance is continuous. If it colors black with silver while the collagen bundles stain yellow, this is because its finer fibers are more easily penetrated by the silver; moreover, with truly elective methods, the staining affinities of the two substances are identical.

Recently, however, by ingenious experiments, Foot<sup>3</sup> has sought to establish the reason for the argyrophilia. He attributes it to a special substance which impregnates the reticulin and which can be extracted by sodium hydrate.

In this paper, we shall describe briefly our observations of the structure of several types of reticulin. We shall then study some new facts relating to the physiochemical constitution of the fibrils of the collagen framework in general, which seem to us to support the unitary hypothesis.

\* Received for publication July 23, 1930.

## RETICULIN OF NERVE, MUSCLE AND FAT

Although the noble elements of these tissues are as different from one another as it is possible to be, their respective reticulins are constructed on exactly the same principle; and this principle is precisely the opposite of that which we have described as governing the relations between the fibroblasts and the framework of the areolar tissue. Instead of submitting passively to the influence of the framework and allowing itself to be modelled by it, as do the fibroblasts, each fat cell, each nerve fiber and each muscle fiber envelopes itself in an extremely delicate and close-meshed reticulum, which constitutes an individual sheath closely applied to it.

These close-meshed, reticulated sheaths spring from branches of fine collagen fibers of a much coarser network that lies between the noble elements, so that the sheaths form a continuous whole even though each sheath is distinctly individualized.

In the *nerve*, the ensemble of the framework forms the endoneurium, the structure of which was described but incompletely by the earlier authors who studied it with silver. Plenck<sup>7</sup> first sketched the details, but these were studied more precisely by Laidlaw<sup>8, 9, 10</sup> with the aid of his excellent method of silver impregnation.

In the *muscle*, the perimysium is constructed on a similar type. It has long been known; we shall not dwell on it further.

We ourselves have studied the *adipose tissue* in thick frozen sections.<sup>11</sup> This technique furnishes preparations that are imperfect as a whole, but in which certain points present images more demonstrative than thin paraffin sections. Fig. 2 represents one of these points. It shows the relations between the protoplasmic wall of the fat cell and the reticulated envelope, and between this individual sheath of the fat cell and the interstitial framework common to the tissue.

In closing the discussion of reticulin under this category, we should point out that in both nerve and muscle the reticulin contains fibroblasts which, as elsewhere, lodge as best they may; whereas each of the noble elements has its own habitation, carefully constructed. In the fat, however, there are no fibroblasts in the interior of the lobules; besides the fat cells, the lobules contain nothing but mast cells and a network of capillaries without any accompanying connective tissue. In order to find fibroblasts, one must search as far as the

On examining preparations of the edematous tumor we see the coarser bundles of collagen ramifying in more slender branches, and these in their turn ramifying in finer and finer fibrils, ending in reticulin. Like the reticulin, the large bundles are arranged in networks. The only difference is that the meshes of the reticulin are so small that they are seen easily in thin sections, whereas the collagen bundles form a network of such large meshes that they cannot be followed in their entire extent even in thick sections; but between the two are all intermediaries.

In sections stained by the selective methods of Mallory and Van Gieson we may establish another fact: all of the collagen fibers and fibrils, when standing alone, stain alike, the intensity depending solely on their thickness. Comparison of these preparations with those of the same tissue fixed in the natural state, where the elements are compressed one against the other, shows that all that which has been believed to be amorphous or fundamental substance is nothing but compact reticulin;<sup>6</sup> but the degree of condensation of the reticulin fibrils, when compressed one against the other, is less than that of the fibrils in the interior of the collagen bundles. Hence it comes about that the so-called amorphous substance appears to be stained less deeply than the bundles. On the supposition that this substance constitutes a primitive phase in the development of the bundles it has been called precollagen, and diminished colorability is given as one of its characteristics. This distinction has no serious basis either in anatomy or in embryology. It is obvious that in embryonic development the more slender forms will appear first, to increase later at all points where there is no reason for them to remain slender; but there is no evidence of a chemical evolution in the substance of the framework except in certain tissues such as bone. In reality, the entire collagenous framework of the areolar tissue is made of one and the same substance which is disposed in fibrils, either free or united in bundles, without any visible trace of amorphous substance, at least not in mammals.

Finally, we should note that in areolar tissue the fibroblasts, even though regularly distributed, do not provoke any change in the framework of their immediate neighborhood; they have no appointed places; they are scattered at random and take the form that is imposed on them by the space which they occupy. In a word, they are modelled by the framework, the structure of which they do not control.

conclusions from them. The change in fuchsinophilia with Van Gieson's method seems to us to be correlated with the progressive change and destruction of the framework, which necessarily begins in the most delicate portions, that is to say, the reticulin of the pulp. That of the trabeculae resists longer because it is more coarse. The connective tissue bundles of the capsule are quite well stained after boiling for half an hour. This is easily understood. In order to prevent their staining by acid fuchsin, the collagen must not only be transformed completely into gelatin but the gelatin must be dissolved and dispersed, which occurs but slowly. In reality, neutral gelatin stains red with Van Gieson, and not yellow as Foot states. It may lose its fuchsinophilia, it is true, but for this change to take place it is necessary that the pH fall below the isoelectric point. No doubt the red staining of the gelatin is less intense than that of the collagen fibers, but this is because it contains more water.

Like Foot, we have found that reticulin, after boiling, is still colored black by silver, but the integrity of the network persists much longer in the liver than in the spleen. After fifteen minutes of active boiling the reticulin of the liver appears relatively well preserved in form and it colors black; but its meshes are smaller, due to the shrinking of the hepatic cells and to the shortening of the fibrils, which contract under the influence of heat exactly like connective tissue fibers. Boiling must be prolonged for half an hour to effect a profound change in the reticulin, in its structure but not in its staining. It swells considerably, trebling the diameter of the fibrils, and these segment into small discs, giving them a transversely striated appearance (Figs. 3, 4, 5). In the spleen, on the contrary, this change occurs as early as the fifth minute of boiling. The reticulum of the pulp has become very incomplete, visible only here and there, but that which remains still stains black. Like the reticulin of the liver, the fibers of the trabeculae and those of the capsule are striated transversely because of their disintegration into discs formed of granules that reduce the silver. After boiling for fifteen minutes or even half an hour, this aspect scarcely changes, but in place of the network fragmented into granules at the beginning of boiling and now having disappeared in form, there remains the débris of its substance which still stains with silver. The splenic reticulin, then, is less resistant to heat than the hepatic reticulin, but despite the changes undergone, the substance of the one, as of the other, con-

delicate connective tissue envelope of the fat lobules. What, then, is the cell that is responsible for the formation of the reticulin?

#### RETICULIN OF THE LIVER AND OF THE SPLEEN

The anatomical arrangement of the reticulated framework of these two organs is too well known for us to describe it anew. We shall say simply that in the liver the reticulin lies in the only free space, the space between the columns of liver cells and the capillaries. In consequence, its orientation may be attributed as well to the one as to the other of these two elements. As for the fibroblasts, they remain cantonned in the portal and subhepatic spaces at such a distance as to render very doubtful their rôle in the fabrication of the framework.

In the spleen there is an obvious connection between the disposition of the reticulated framework and that of the cells of the splenic parenchyma. In the malphigian bodies the lymphocytes influence the network rather as a group. In contrast, the cells of the pulp are individually enshrouded in the meshes of a very regular network which is moulded on them. Its fibers present a remarkable feature; they are finely and uniformly wavy, so much so that they resemble spirochetes, this form being doubtless connected with the great variations in volume of the spleen under physiological conditions. Here, too, the cells peculiar to the reticulum, which represent fibroblasts, have no influence whatever on the orientation of the framework, this being governed entirely by the cells of the parenchyma.

These organs especially are favorable objects for the study of the changes made in their substance by the different treatments that may be applied. Foot has the merit of commencing studies in this field. We have merely sought to verify his results, but our conclusions differ very much from his.

In the first place, we find ourselves differing from our colleague on certain points of technique. This question will be discussed in another paper. We shall say here that, contrary to Foot's statement, the reticulin can be impregnated perfectly in black and without difficulty in frozen sections of fresh organs, with no other fixation than that resulting from the passage through the ammoniacal silver bath.

As to the results obtained on boiling the spleen, our experiments confirm those of Foot in a general way, but we do not draw the same



N:500 HCl, others in pure water. In these fluids, they expelled almost the entire quantity of their protoplasm in the form of white clouds, falling slowly to the bottom of the receptacle. After complete neutralization they were fixed in formol, or Zenker's or Helly's fluid, embedded in paraffin and cut in thin sections; with silver, the reticulin colored a perfect black (Fig. 6). The argyrophilia of the reticulin, then, cannot be due to an argyrophil substance impregnating the fibrils and susceptible of removal or destruction by the action of 4 per cent sodium hydrate for twenty-four hours.

Stained by Van Gieson's or by Mallory's method, these sections, almost completely freed from cells, show the reticulin stained selectively with perfect clearness, whereas in ordinary sections its existence would scarcely be suspected. It is well known that an analogous procedure, more appropriate for histological research, was used by Mall, who resorted to pancreatin to rid the tissue of the confusing cells and to reveal the reticulin in an excellent manner at a time when silver impregnation was unknown.

#### THE RÔLE OF PHYSICAL PROPERTIES IN SILVER TECHNIQUE

The achievements as well as the defects of silver technique depend on this, that they rest on physical properties rather than on chemical affinities, as we shall now show. In a section of connective tissue colored with silver, the large bundles are yellow, the smaller bundles are darker as they become smaller and the finer fibrils are black. It is said currently that the parts of the connective tissue network that stain black are argyrophil, which would imply that the parts colored yellow are argyrophobe; but this selectivity of the two colors is far from being invariable. Varying with the fixative and the slightest details of technique, the black is seen to encroach more and more on the yellow, and indeed it is not difficult to stain all black. Thus Laidlaw's method<sup>8, 9, 10</sup> for the peripheral nerves colors the pia mater exactly like the individual reticulated sheaths of the peripheral nerve fibers. The color of the reduced silver, then, so far as it concerns the elements of the connective tissue framework, is a relative and not an absolute property.

Coloring by silver depends on the formation of a colloidal sol which fixes itself on the constituents of the tissues, themselves also

tinues to color black with silver as long as it is not completely disintegrated.

In short, staining by silver and by acid fuchsin both show progressive alteration of the framework, ending in its destruction; but silver enables us to see the débris of the reticulin as long as it exists, whereas fuchsin does not. Is there anything surprising in this? The Van Gieson stain reveals almost nothing of the intact framework of the liver or of the splenic pulp as long as the cells of these organs remain in place; and the staining power of the fuchsin on reticulin diminishes necessarily when the proteid matter of the fibrils swells and is diluted by the absorption of water in boiling. The swelling may be estimated as at least twenty times the original volume and the intensity of the staining per unit volume must be decreased in the same proportion. In coloring by metallic impregnation, on the contrary, there is no necessary relation between the volume of the colloidal silver precipitated and the quantity of the proteid molecules. After boiling, the swelling of the fibers, augmenting their permeability, may favor the accumulation of the metal and produce the opacity despite the absorption of water. Thus the contrast described by Foot between the conservation of the argyrophilia and the diminution of the fuchsinophilia in boiled reticulin finds a natural explanation and there is no need of any hypothesis.

Furthermore, it is obvious that boiling, which fragments the reticulin, changes its substance much more than does sodium hydrate which, acting for twenty-four hours, causes no morphological change in the reticulum, does not destroy the fuchsinophilia, as Foot has proved, or its argyrophilia, as we shall show. In fact, concerning the disappearance of the argyrophilia after treatment with sodium hydrate, we are obliged to contradict Foot's statements.

Slices of liver and spleen of dogs, about 5 or 6 mm. thick, were immersed for twenty-four hours in normal sodium hydrate (4 per cent), washed thoroughly until neutral, fixed in Helly's fluid and frozen sections made. The reticulin impregnates black without the slightest difficulty.

In another experiment, small pieces of dog's liver, 2 mm. thick, were suspended from tiny glass hooks which served to transport them to the different fluids without exerting the least mechanical action on them. After twenty-four hours in the sodium hydrate, where they became white and very soft, some were immersed in

The penetration of substances dissolved in a heterogeneous colloidal medium is an extremely complex phenomenon. At each passage from one phase to another, there occur reactions which modify their distribution on the one side or the other of what are known as the interfaces, and these reactions may influence the reduction of silver in the silver techniques, which would explain the accumulation of colloidal precipitate in these interfaces. As we shall see presently, this is easily demonstrated. There are other properties of the interfaces, however, which perhaps play a still more important rôle in the process under consideration.

We would speak of the phenomena connected with the radius of curvature and consequently the surface tension, which increases rapidly as the dimensions of the object diminish. Quincke has calculated the conditions of equilibrium in cells of various dimensions, all other conditions being equal. For spheres of radii of 10 microns, 1, and 0.08 micron, the interior pressure must exceed the external pressure by 0.046, 0.46 and 5.75 atmospheres respectively. With collagen fibers, which have no semipermeable membrane, it is not the osmotic pressure that intervenes, as with cells, but the pressure of imbibition. Practically it amounts to the same thing, for, in the swelling of colloids, the water is absorbed much more in proportion than the salts.

To the differences in internal pressure are added necessarily differences in the electric phenomena in the interfaces; for these phenomena also depend on the size of the particle. We know the considerable rôle of electricity in colloidal phenomena and consequently in the methods of silver impregnation.

In support of what we have just said on the importance of the interfaces, we shall present two orders of facts: (1) the manner in which fibrin behaves toward silver according to the medium in which the fibrils occur; and (2) the constitution of the silver sol in the connective tissue preparations as revealed by the ultramicroscope.

#### CONDITIONS UNDER WHICH FIBRIN COLORS BLACK WITH SILVER

Fibrin formed in the tissues passes for non-argyrophil. We have not yet sufficiently studied this question, which the variability of fibrin makes very complex, to have a personal opinion on this point. This much is certain, that the thin clot of citrated plasma formed on

in the colloidal state. In the first place, we know that the color and opacity of a metallic sol depend on the dispersion of the metal, that is, the dimensions of its particles, much more than on the quantity of metal in suspension. In the second place, hydrophil colloids, that is to say, albumins, and in consequence the substances composing the connective tissue framework, have a permeability which varies much more according to their hydration than according to their chemical constitution. The composition of the baths, their concentration, the proportion between the silver salt and the reducer, all play a capital part in the degree of dispersion of the colloidal silver which is produced in the course of the reduction.

If these factors should operate in a homogeneous medium, perfectly permeable to all the substances employed, the resulting color would be uniform; but collagen, even supposing it to have an invariable chemical composition, is far from being homogeneous and its permeability is not invariable. Being itself colloidal, its dispersion may vary from one point to another; and it is arranged in fibrils which are sometimes free and merely alongside of one another, sometimes massed in dense bundles. The fibrils of a connective tissue bundle are not associated accidentally and temporarily. The bundle is a stable anatomical unit which does not dissociate in the edematous tumor when all the fibrils of the reticulin separate one from another. What is the cause of this stability? We do not know exactly but, as we shall show presently, there are reasons for believing that around each bundle there is a membrane, too thin to be demonstrated and yet capable of modifying the permeability of the whole bundle; and that, in the interior, the fibrils are more or less agglutinated, with what substance we are unable to see. These are conditions which differ physically very much from those in which the fibrils of reticulin find themselves.

Permeability is a very important factor. It affects the concentration of the penetrating reagents, probably more that of the reducer than that of the silver. In Cajal's method, the periphery of the piece is black, even if it has been cut before immersion in the reducer. Toward the center it is paler and paler yellow, more especially because the reducer arrives there more and more diluted. Moreover, in the zone where the general color is yellow, the large axons are yellow while the small ones are black. This is exactly what happens among connective tissue fibers colored with silver.

coarser reticulin such as that of the liver of the larger animals, the fibrils are bordered on each side by a bright line but the central portion remains dark.

With higher magnification all of these lines appear finely granular, even when the impregnation is very pure and when the filaments appear smooth by transmitted light. It is obvious that this appearance is due to a multitude of tiny reflecting surfaces. In the yellow areas the silver granule is smaller, often invisible; the aspect is that of a submicroscopic sol and the reflection by the surface of the collagen bundle is similar to that of a smooth surface. The yellow, transparent silver, then, is more highly dispersed than the opaque silver which appears black by transmitted light because of its opacity.

The blackening of the fibers is partly due to a thick deposit of silver on their surface, for their diameter seems greater when colored with silver than by other methods. Moreover, on examining coarse fibers, those of the liver for instance, with high magnification and transmitted light, we see that their center is not absolutely opaque. If the color were due exclusively to a uniform impregnation of the entire thickness of the substance, such an appearance would not be produced. A metallic deposit on the surface of a filament is the result of physical attraction and not of chemical combination with the substance of the filament.

On the surface of the yellow collagen bundles, by transmitted light, we see nothing indicating a deposit of silver. This deposit exists nevertheless, for it reflects in the ultramicroscope, but it is too thin and too transparent to be seen by direct illumination. It is even possible that it is this thin veil of silver on the surface of the yellow objects that gives to the images in sections stained by Cajal's method, even when they are very thick, that perfect definition of the finest details, often noticed, which no other technique has attained.

In the course of a silver impregnation we may concede two successive phases: (1) colloidal silver is produced as a chemical phenomenon in the reagents; (2) it localizes and fixes itself by a phenomenon of election which rests on the physical properties of the tissues. Obviously, the physical properties of a body depend on its chemical constitution but only in a certain measure. There is no question that different bodies may realize equivalent physical conditions and, on the contrary, the same body, according to circumstances, may acquire different physical properties. It follows that various sub-

a slide by adding a drop of a solution of calcium chloride to a drop of plasma, washed to remove the albumin of the serum and fixed in Helly's fluid, impregnates an opaque black just as well as does reticulin (Fig. 7). However, if we use the spontaneous clot removed from a centrifuge tube in which it has formed, wash it superficially, fix and embed in paraffin, the result is quite different. At the periphery, in the thin layer that has been affected by the washing, the fibrils impregnate black, the better as they are nearer the surface; in the center is seen nothing but granular matter uniformly colored brown, without the least trace of fibrils. Nevertheless fibrils exist at the center as well as at the periphery, for phosphotungstic acid hematoxylin brings them out very clearly. A change in the medium outside of the interface, then, suffices for the material inside of it, the fibrin, to manifest an argyrophilia that had been completely masked.

#### ULTRAMICROSCOPIC STUDY OF SILVERED PREPARATIONS

The ultramicroscope, the instrument *par excellence* for the study of colloids, enables us to see interesting aspects of silvered preparations, both those stained by Cajal's method and those treated by the techniques derived from that of Bielschowsky. In sections mounted in balsam, all luminous phenomena due to the hydrophil colloids of the tissues vanish, leaving visible those belonging properly to the colloidal substances introduced by the fixative or by the staining fluid, and especially to the colloidal silver that is fixed on the anatomical elements. In this way we may study the metallic sol, recognize the degree of fineness of the granules, which varies according to the territories where deposited, and learn the peculiarities of its elective distribution.

With transverse illumination of a preparation colored with silver, the reticulin is brilliantly illuminated in white, sometimes also in yellow, in a dark field. The collagen bundles, on the contrary, when they have been colored yellow in the preparation are illuminated diffusely, though permitting their fibrils to be seen more or less; but when the light is regulated carefully, their borders give a bright reflection of yellow tint. It is obvious that their surface is mirror-like (Fig. 8).

In a fine reticulin, such as that of the edematous tumor or of the splenic pulp, each filament appears as a simple brilliant line. In

succeeded in obtaining a connective tissue framework, have confirmed this absolutely,<sup>12</sup> and it had already been demonstrated by the methods of pure histology. What cells have the power of invoking the appearance of a connective tissue framework? Fibroblasts inhabit almost the entire extent of the framework, and it is quite natural to believe that they play the principal part in its formation; but we must not be too exclusive. In adult tissue, we have already shown the intimate relations existing between reticulin and various cells in territories where fibroblasts exist and also where they do not exist. This is much more obvious in the embryo where certain dispositions of the framework around the notochord (von Ebner, Klaatsch) and in the cornea (Kessler) cannot be explained reasonably if one would reserve to the fibroblasts alone the privilege of fabricating the interstitial substance. In reality, many kinds of cells are capable of collaborating in the construction of the connective tissue edifice. What reasons have we for believing, therefore, that all of these collagens of diverse origins are identical?

Let us leave to one side the question of the multiple origins of collagen which will occupy us later on; let us not insist on those instances where the connective tissue plays an accessory rôle of interstitial framework for the noble elements of the viscera, of the muscles, of the nerves or merely of the subcutaneous fat. Let us limit ourselves to the forms in which of itself it constitutes a part of the organism and where as fixed cells it contains only fibroblasts, the derma, the subcutaneous areolar tissue and the tendons. Among these different forms of collagenous tissue we find considerable differences, not only of texture but also in the dimensions and grouping of the fibrils, the transparency or, on the contrary, the reflecting property of the bundles, their aptitude to swell—in a word, physical variations so great that we must be tempted to suppose them bound to chemical variations in spite of the uniformity of their staining affinities.

These are the *tissue variations* of the collagen framework which end in the formation of different connective tissues; but each one of these tissues, in its turn, may not be identical in all regions of the organisms. This fact is obvious for the derma and we shall give presently a remarkable example for the tendon tissue. There are, then, *regional variations* which superpose themselves on the tissue variations.

stances may impregnate black while the same substance, of the collagen group, may impregnate either in black or in yellow according to circumstances. The argument in favor of the duality of substance, based on the different color taken by reticulin and by the collagen bundles, is of no value.

### PHYSICOCHEMICAL CONSTITUTION OF THE COLLAGEN FRAMEWORK

What we have just said does not mean that collagen is identical in all vertebrates, nor that in animals of the same species it is identical in all parts of the connective tissue framework; it means simply that histological methods are impotent to reveal the variations which logically one might suppose to be present.

Like all albumins, collagen is a polypeptid. We know that these substances vary infinitely according to the number and kind of their constituent amino-acids, the manner of union of the radicles, the polymerization of the molecules. Among the albumins there are classes characterized by certain primordial properties; among these classes there are groups possessing secondary properties in common, and so on as far as the ultimate variety which differs from its neighbor only in an insignificant detail, and which cannot be distinguished with certainty by any means at our disposal.

A priori, it is quite probable that collagen has not a uniform composition in the whole extent of the connective tissue framework, and it is certain that it varies from one species of animal to another. In the first place, what is collagen? We have reasons for believing that it is a complex formed by a certain kind of protein with neutral salts of metals that exist in the animal economy. Here already is a cause of variability, for these salts are many and it is not unreasonable to suppose that each connective tissue fiber, whether of reticulin or of a collagen bundle, may consist of a mixture of molecules differing one from another by the salt which they contain, the proportions of the mixture differing from one organ to another. The albumin may vary also.

We know that the connective tissue substance appears between the cells, in contact with them but not by the exfoliation of a so-called edexoplasm which has been supposed to detach itself from their substance. Maximow's observations in tissue cultures, in which he



tissue, present great interest from the general point of view. They show that the specific activity of homologous cells is everywhere subject to the influence of a regulation of a superior order. It is the entire organism, an energy system, coördinated and equilibrated in all of its parts, which controls the quality as well as the quantity of the substances elaborated by each one of its elements: it matters little whether these products are intracellular or intercellular. However, by its very generality, this principle ceases to be applicable when we are searching for a specific difference between reticulin and the collagenous substance which are mixed intimately in the same tissue.

In fact, it is probable that reticulin is not identical everywhere. We have shown that the reticulin of the spleen, for example, behaves differently from that of the liver under the influence of heat. Does this result from different properties or merely from greater fragility due to the delicacy of the splenic reticulum? Or from the action of substances expelled from the cells by the heat? Of this, we can assert nothing.

Since Mall's time, it has been believed that the great characteristic distinction between collagen and reticulin is the yielding or non-yielding of gelatin. We have tried to verify Mall's statement that the action of pancreatin usually prevents the formation of gelatin and have found that tendons digested for twenty-four hours in the incubator, in a solution of pancreatin of Mall's formula and freed from all cellular elements, yield gelatin on boiling as easily as fresh tendons. Moreover, in those organs where the authors find no gelatin, it may be that the gelatin is merely made soluble in the cold and consequently prevented from jelling by the action of substances coming from the parenchymatous cells, the mass of which is infinitely greater than that of the reticulin, and that for this reason the presence of gelatin was overlooked.

Suppose that we should succeed in eliminating this error and demonstrate to a certainty that, as far as the production of gelatin is concerned, the framework of a viscus is different from that of a given connective tissue; still this does not suffice to establish a specific difference between the two substances, *collagen* on the one hand and *reticulin* on the other. To reach this conclusion, it is necessary to prove a difference between the chemical constitution of collagen and reticulin in the same tissue, the areolar tissue, for instance:

Must we seek then among the fibroblasts for special varieties endowed with different potentialities as the first cause of all these anatomical, physical and probably also chemical variations of the collagen framework? Scarcely. When dead tendons are grafted in areolar tissue, the graft attracts the fibroblasts from the surrounding tissues, and at the same time that it is vacularized it is repopled completely, to the point that it resumes definitely the properties of living tissue. The fibroblasts of the areolar tissue change their form when they penetrate the tendinous framework and assume immediately all the characteristics of tendon fibroblasts.<sup>13, 14, 15</sup> In our opinion, this experiment shows that even those varieties of fibroblasts that are most dissimilar in appearance possess in reality the same powers. Hence we are led to consider the different forms of collagenous framework as conditioned essentially by the place where they develop in the organism and by the totality of the interrelations which govern in the territories which they occupy.

As far as collagen is concerned we can distinguish these diverse substances produced by cells of the same species only by differences in certain physical properties; but there exist other and more favorable substances where it is easy to present evidence of chemical differences. As an example we shall take the regional variations of a substance entirely different from the albumins, but which lends itself particularly well to the demonstration. The subcutaneous white adipose tissue is everywhere similar, in appearance at least, but already the microscope shows that the fat cells, otherwise specifically identical, have different dimensions according to the different regions of the body. In *Delphinus tursio*, Margaillan<sup>16</sup> has shown that, even though the contents of the fat cells everywhere consists of oil, it differs none the less in chemical composition from one territory to another. These chemical territories are more or less extensive (in the head, for instance, there are three very small ones) but their anatomical delimitation is invariable. Moreover, the different chemical territories are not scattered in a disorderly manner; the variations observed in the chemical composition of the oil follow a systematic order in the entire extent of the subcutaneous fat. In a word, there is a chemical architecture of the fat comparable to the cyto-architectonic and the myelo-architectonic of the cerebral cortex.

Chemical variations of this kind, which are established with certainty for the fat and which probably exist also in the connective

ing in a collodion sac. The liquid then takes the form of a limpid jelly which melts irreversibly about 50°C. If, instead of heating this jelly, it is acidified again, it is redissolved and becomes again capable of forming a fibrous clot by the action of neutral salts.

The dissolved substance is the albumin of the collagen, its salts having been removed by the reversible action of the acid. If the acid is removed by dialysis and the dissolved salts at the same time, the albumin jells; but if, without removing the acid, a sufficient proportion of salt is added, there is produced a mass action, the result of which is the reconstitution of the complex albumin plus neutral salt, and the reappearance of the collagen in its typical form, which is fibrillar.

These phenomena were long studied by one of us in a series of notes.<sup>17, 18, 19</sup> We shall repeat here only what can contribute to the discussion of reticulin.

In order to observe the formation of the clot under the microscope, two thin, narrow streaks of paraffin should be traced with a hot iron along the edges of a slide. A small drop of the tendon solution is placed in the middle and covered with a cover glass, which is cemented along the borders that rest on the paraffin. The drop, flattened between the two glasses, should be free all around its margin. The free space is then filled with a 1 per cent solution of NaCl. The drop disappears but soon there is formed in its place the reconstituted clot of collagen which can be studied with the ultramicroscope. It may be fixed also in neutral formol, in Bouin, Zenker's or Helly's fluid after washing in water to remove all traces of acid; then separate the two glasses and treat the layer of clot adherent to each of them like any mounted section.

It is then seen that the clot is formed of fibrils, the thickness of which varies in different preparations. These fibrils are simple and arranged in feltworks, or they may ramify and anastomose with one another in the form of networks with closed meshes which may strangely resemble reticulin (Fig. 9).

In favorable preparations, with the aid of the ultramicroscope, details may be seen in the thickness of the coarser fibers, aspects that indicate the endogenous formation of secondary fibrils inside of the primitive fibrils, all crossing and growing more intricate by intussusception. In our opinion, this throws light on the mode of formation

We have reason to believe that the substance of the reticulin of areolar tissue, which is very abundant, does not differ from that of the collagen bundles. If, after having spread on a slide a frozen section of the unfixed edematous tumor and applied a cover-glass, the preparation be heated along a narrow line by an electric current passing through a wire applied to the under surface of the slide, the section is seen to divide suddenly into two parts as soon as the temperature reaches a certain degree. The reticulin and the collagen bundles liquefy at the same moment.

To summarize, we are led to conclude that, extensive as we may suppose the tissue and regional variations of the chemical constitution of the different elements of the connective tissue framework to be (the elastic fibers excepted), there is not at present any reason for believing that these differences can furnish a means of separating two types, systematically distinct although intimately mingled.

#### THE ARTIFICIAL PRODUCTION OF COLLAGEN FIBERS IN FELTWORKS, OR IN A RETICULUM WITH CLOSED MESHES

Under the microscope, with transverse illumination, it is easy to watch the formation of an artificial fibrous clot of collagen. The fibrils appear in the form of extremely fine filaments, scarcely perceptible, which, in a few minutes, elongate, thicken and become luminous at the same time that their structure seems to become more intricate.

To observe these phenomena, it suffices to treat the tendons of a rat's tail for twenty-four hours with a dilute acid, acetic acid 1:25,000, for instance. The tendons are obtained by beginning at the tip of the tail and tearing off pieces 1 cm. long. One tail yields about 70 cg. of tendons, which are placed in a dish containing 50 cc. of the acetic acid solution, taking care to disperse them well throughout the fluid. If, after half an hour, there is not much swelling, change for fresh fluid. In twenty-four hours the tendons will be much swollen and very fragile. The solution is filtered through several thicknesses of gauze. It is limpid, slightly viscid, and contains a little more than 1:1000 of dissolved substance.

An opalescent fibrous clot can be produced in a test tube by adding to this fluid solutions of neutral salts of various concentrations (NaCl from 4 to 20 per mille); or the acid may be removed by dialyz-

over, all the tendons of the rat, the rabbit and the dog, when treated with sodium hydrate and a dilute acid, yield Collagen B; but the tendons of bovines have been completely refractory up to the present time. Whether treated by an acid or by sodium hydrate, they swell and become transparent; if the action of the sodium hydrate is prolonged, they dissolve but they do not at any time yield substances coagulable by neutral salts.

We do not conclude from this that the different tendons are made of specifically different substances but merely that there are varieties in the chemical species *collagen*. While presenting many features in common, these varieties may be distinguished by properties just as striking as those on which many authors rely to make two distinct species of reticulin and collagen.

### SUMMARY

1. There exist different varieties of reticulin, all in continuity of substance with the collagen bundles in such manner that the connective tissue framework forms a single system. The elastic fibers, on the contrary, though mingled with the connective tissue framework, have no continuity with it.

2. The subcutaneous areolar tissue is well adapted to complete histological analysis. Thanks to the procedure of the edematous tumor and contrary to the general opinion, it can be demonstrated that the collagen bundles form a network like the reticulin. From the network of reticulin, which in this tissue is particularly delicate, to the network of collagen bundles, the transition is gradual and continuous, for the thickness of the fibers as well as for their coloring by silver.

3. When, in a tissue provided with a connective tissue framework, there are fixed cells other than fibroblasts, it is these cells that govern the arrangement of the reticulin. The fibroblasts, on the contrary, are scattered at random; they lodge where they can and they do not cause to be constructed around themselves any special arrangement.

4. In the adult, the fibroblasts people the connective tissue framework in almost its entire extent, but they are absent in some territories, such as the fat lobules and the hepatic lobules, where the framework is exclusively reticulated.

of collagen bundles. We may thus explain the relations of the fibers to one another in these bundles and also their enveloping membrane, the existence of which we have been led to suppose from the mirror that forms on the surface of the silvered sections.

We shall call the substance of this artificial clot Collagen A. In polarized light, its fibrils have the same birefringence as those of natural collagen. Like the latter, they may be fixed and stained by selective methods. They take the anilin blue of Mallory, the acid fuchsin of Van Gieson; they stain red with phosphotungstic acid hematoxylin. Finally, although their substance was derived from tendon, they are as "argyrophil" as the fibers of reticulin itself, for they impregnate easily in opaque black. Collagen A is certainly very close to the natural collagen. It differs only in its greater solubility in dilute acids, probably due to a slight polymerization.

Only the tendons of the rat's tail give Collagen A. The other tendons swell without dissolving. However, another substance, differing from Collagen A but also coagulable in fibrils by the action of neutral salts, may be extracted from the tendons of the rabbit and the dog. We shall designate this substance Collagen B. The technique is as follows: The tendons are left to swell for twenty-four hours in normal solution of sodium hydrate (4 per cent). They are then washed to the point of neutrality and treated with dilute acid, as already described. There results a fluid which, treated as before, yields a fibrous clot. This clot differs from Collagen A in that its fibers are finer, tortuous, always ramified and anastomosed in closed networks of peculiar aspect. Moreover the clot is fragile, but when treated by the selective histological methods it stains exactly like the clot of Collagen A and it also impregnates perfectly black with silver (Fig. 10). Here then is a substance which, after having resisted sodium hydrate, is yielded by the tendon when treated with dilute acids and which is no less argyrophil than reticulin itself.

These experiments show that it is useless to try to distinguish reticulin chemically from collagen by the color that it takes with silver. They show also how much homologous substances, though colored alike by the same technique, may differ when submitted to other methods of investigation. Under the action of dilute acids, the tendons of the rat's tail behave differently from the tendons of the paws of the same animal and the tendons of other animals. More-

nature to establish a distinction between a collagenous system and a reticulin system; for nothing has been observed so far that warrants the supposition that the collagen part and the reticulin part of a given tissue differ in any way in the chemical constitution of their substance.

For the translation of this memoir, we have profited by the kindness and competence of Dr. George F. Laidlaw. We offer him our cordial thanks.

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5. Treatment of the tissues with normal solution of sodium hydrate (4 per cent) does not hinder the impregnation of the reticulin in opaque black by silver.

6. Boiling the tissues, which causes progressive fragmentation of the reticulin, does not prevent the coloring of the fragments with silver as long as they persist. The reticulin of the pulp of the spleen does not resist boiling as long as that of the liver.

7. In silver impregnations, the color assumed by the fibers depends on physical conditions, on their thickness and conformation, but not on their chemical composition. This feature, then, cannot serve as a criterion to establish a systematic distinction between collagen and reticulin. The interfaces play an important rôle. Viewed with the ultramicroscope the collagen bundles present a broad, mirror-like surface, while the reticular fibers appear as sparkling lines.

8. Like reticulin, fibrin stains black with silver, on condition that the clot has been washed before fixation, but it does not stain if the albumins of the serum remain in contact with the fibrils.

9. Authors state that concentrated decoctions of viscera submitted to prolonged boiling do not jell on cooling. This result, the chief argument for the dualistic hypothesis, may depend on several causes; even if it does not rest on a parasitic phenomenon, in itself it is no more important than other variations observed in the attributes of the connective tissue proper, according to the tissue or the region involved.

10. Treated with a dilute acid, the tendons of the rat's tail, but not those of the paws, yield a substance (Collagen A) which coagulates in fibrils under the influence of neutral salts. These fibrils have all the properties of natural collagen except that they are more easily soluble in acids; they stain black with silver exactly like reticulin. The tendons of the rat and those of the rabbit and the dog, treated with sodium hydrate and then with a dilute acid, yield another substance (Collagen B) which also coagulates in fibrils with neutral salts. These fibers also stain black with silver. Tendons of bovines yield neither Collagen A nor Collagen B.

11. To say nothing of differences in the zoölogical series, homologous substances in the same animal present regional variations that selective stains do not reveal. Variations of this kind exist between the reticulins of various organs, but these differences are not of a



## DESCRIPTION OF PLATES

With the exception of Fig. 2, all the figures are photographs without retouching. All of the preparations were colored with ammoniacal silver.

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### PLATE 121

FIG 1. Artificial edematous tumor of the abdominal subcutaneous tissue of the rat. The reticulin is black. The collagen bundles in ribbon form are light yellow in the section, scarcely tinted in the photograph.

Fixation: bichromate-formol-uranium (Tupa's fluid). Celloidin section, 50 microns thick.  $\times 250$ .

FIG. 2. Reticulin of the adipose tissue. Reticulated sheath of the fat cells. Some of the cells have been sliced by the knife and show their thin protoplasmic wall beneath the reticulum. Loose-meshed intercellular reticulum.

Fixation: formol-bromid. Frozen section, 100 microns thick. Drawn with camera lucida.

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PLATE 122

FIG. 3. Reticulin of intact horse liver.

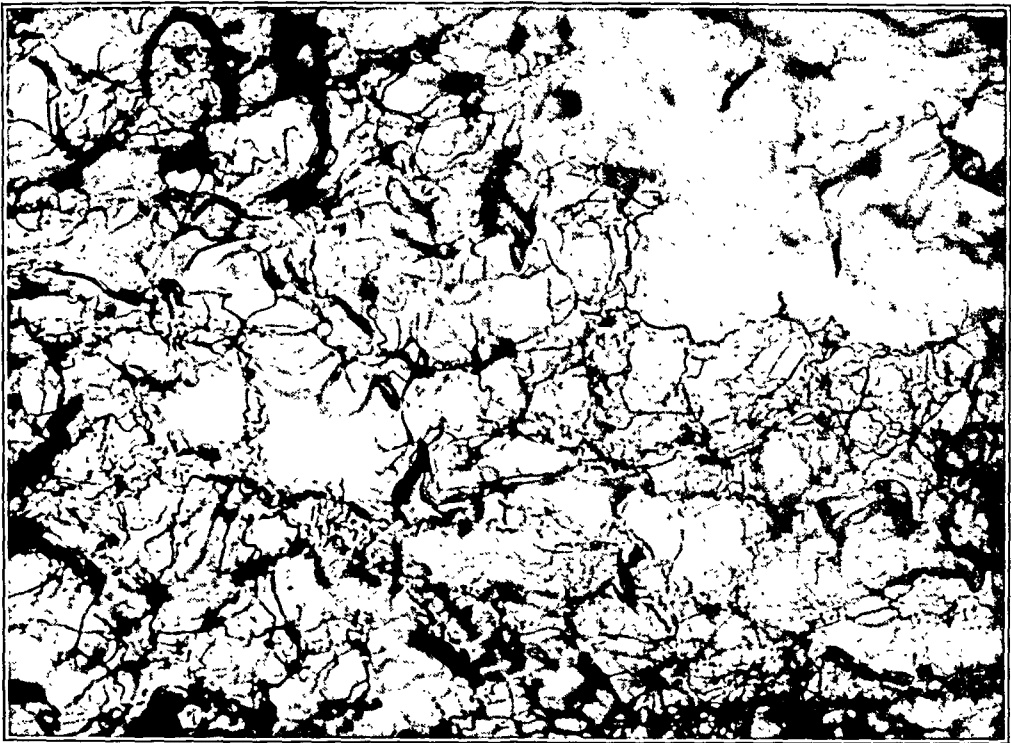
Fixation: Zenker's fluid. Paraffin section, 10 microns thick.  $\times 500$ .

FIG. 4. Same liver after boiling one-quarter of an hour.

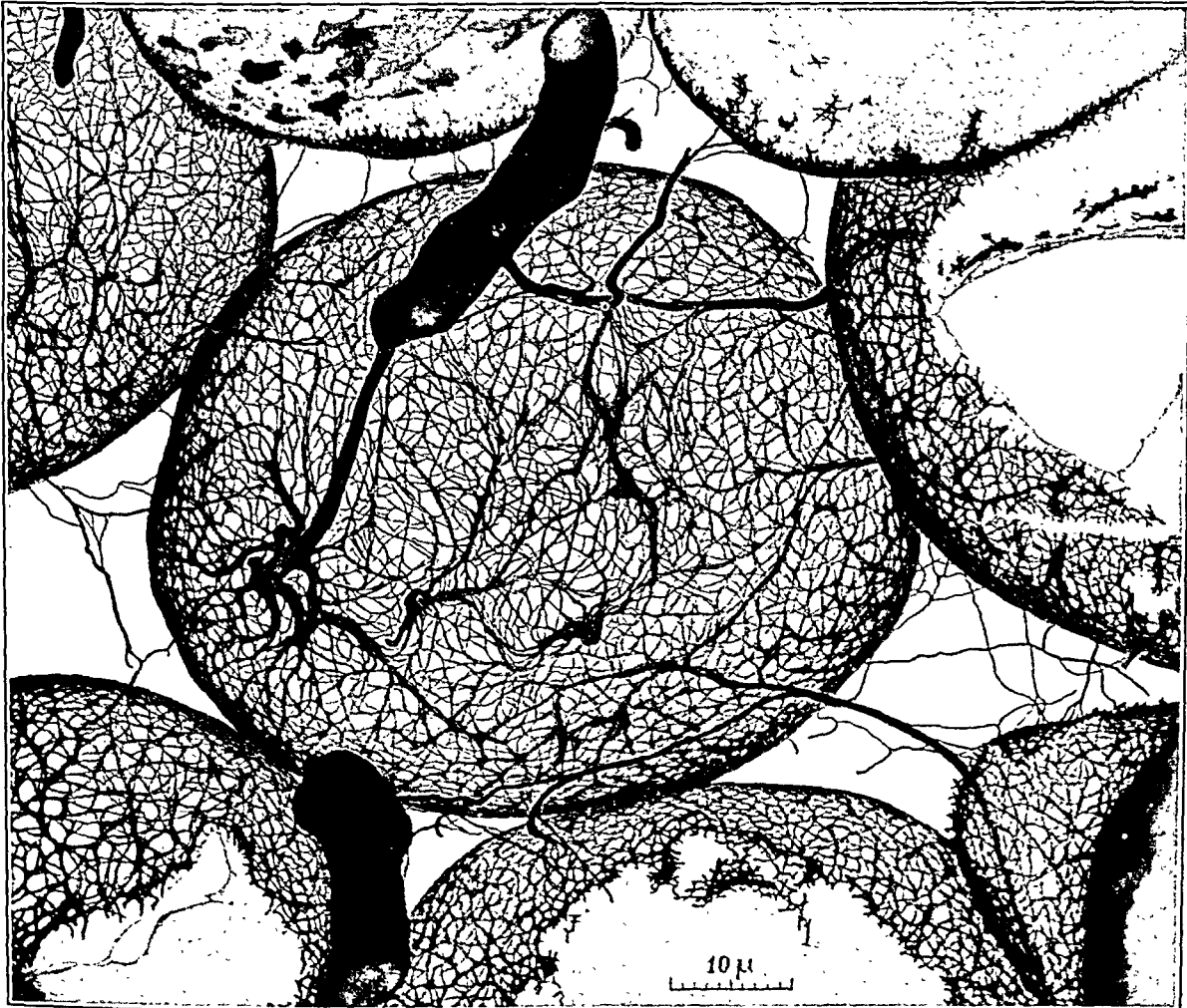
FIG. 5. Same liver after boiling half an hour.

FIG. 6. Dog's liver treated with 4 per cent NaOH for 24 hours. The hepatic cells have disappeared. The reticulum is intact morphologically and colored black.

Fixation: Helly's fluid. Paraffin section, 15 microns thick.  $\times 500$ .



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PLATE 123

FIG. 7. Thin fibrinous clot of citrated rabbit plasma, formed on the slide by adding a solution of  $\text{CaCl}_2$ ; washed; fixed in Helly's fluid.  $\times 500$ .

FIG. 8. Capsule of horse liver. Reflection from the surface of the collagen bundles, colored yellow in the preparation. The light is regulated so that the interior of the bundles is not illuminated and remains absolutely black. Nothing is seen but the reflection from their surface.

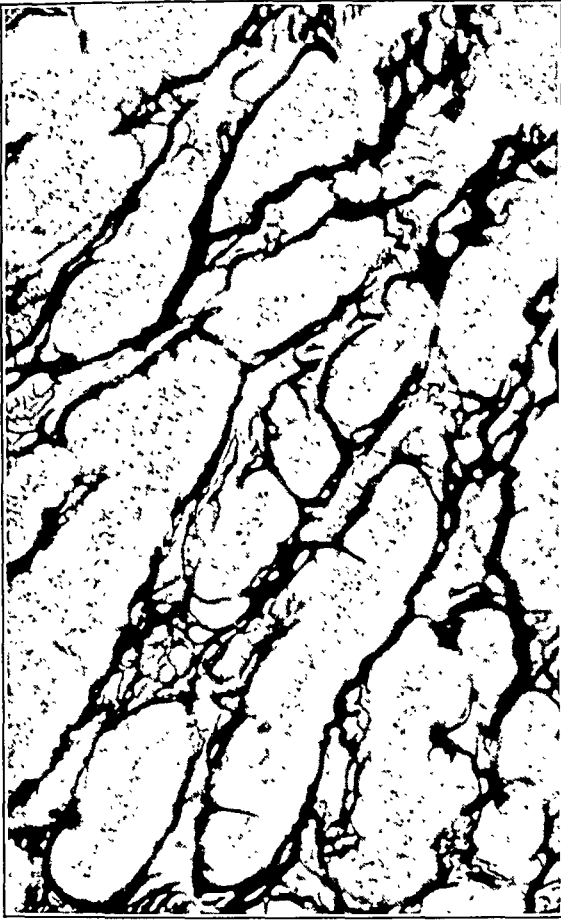
Fixation: Zenker's fluid. Paraffin section, 10 microns thick. Ultramicroscope.  $\times 400$ .

FIG. 9. Artificial clot of Collagen A (tendons of rat's tail), formed between slide and cover-glass by mixing the acetic acid solution with 1 per cent NaCl.

Fixation: neutral formol, 10 per cent.  $\times 500$ .

FIG. 10. Artificial clot of Collagen B (tendons of rat's tail), treated first with 4 per cent NaOH, then by HCl, N:500. Same technique for the coagulation.

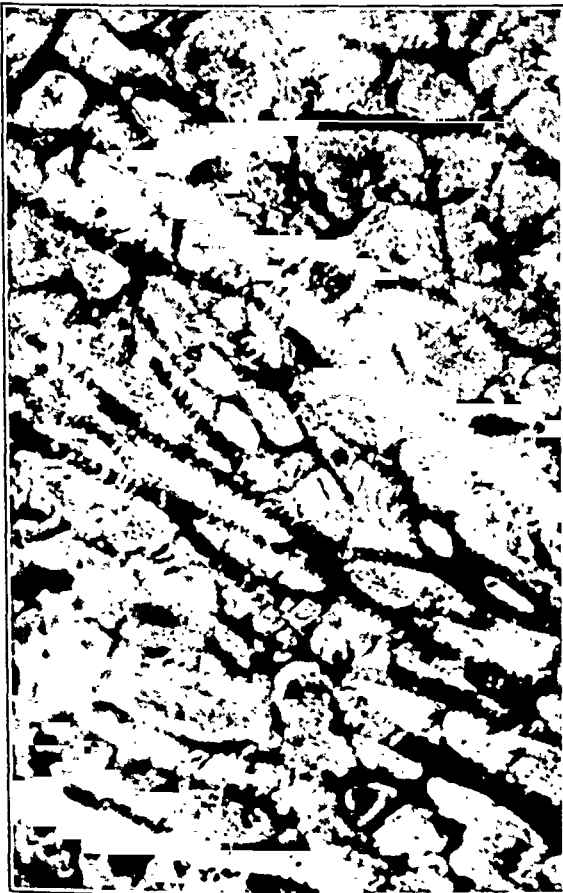
Fixation: Helly's fluid.  $\times 500$ .



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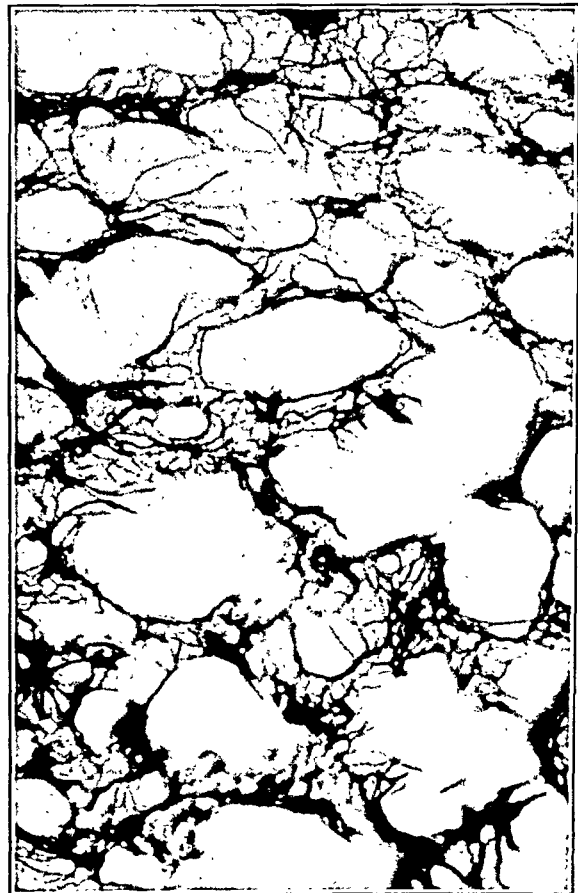


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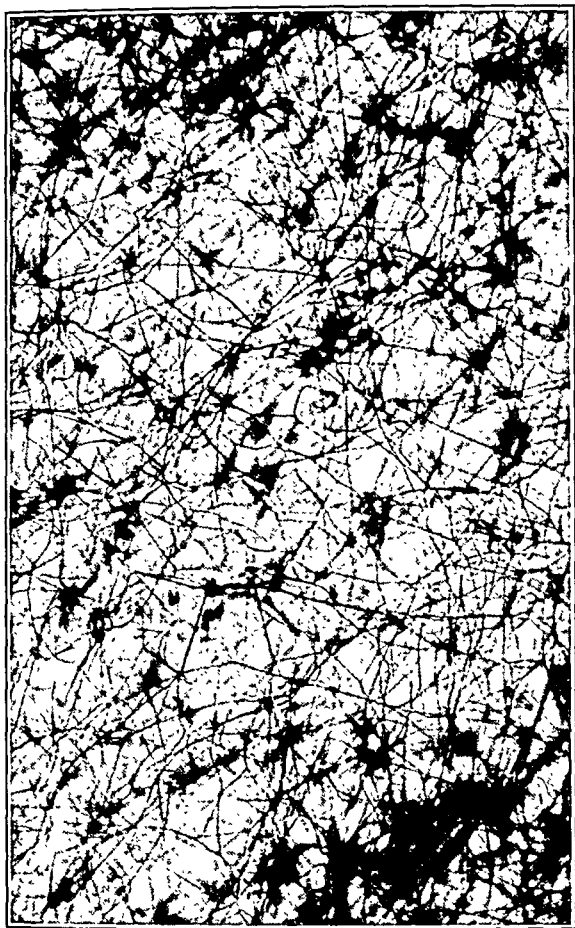
Nageotte and Guyon



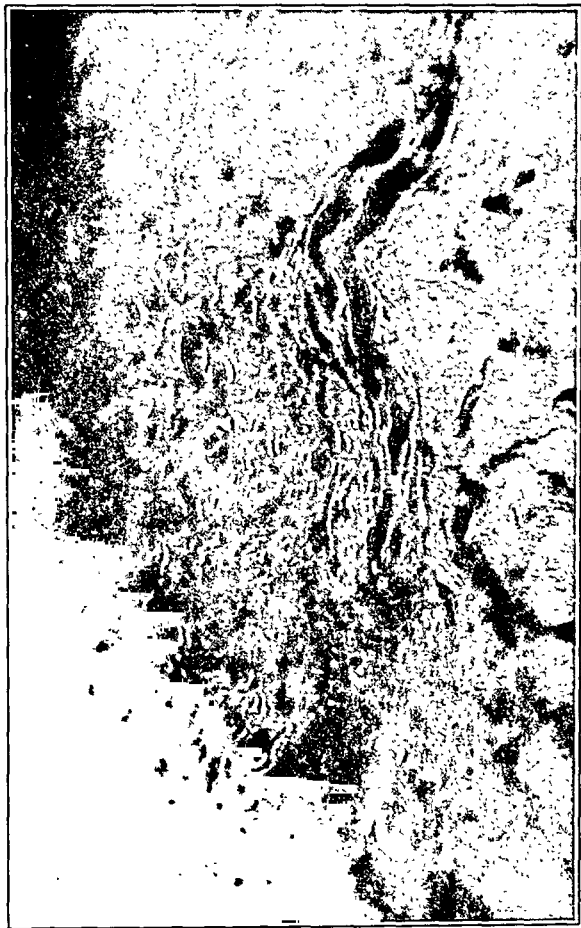
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Reticulin





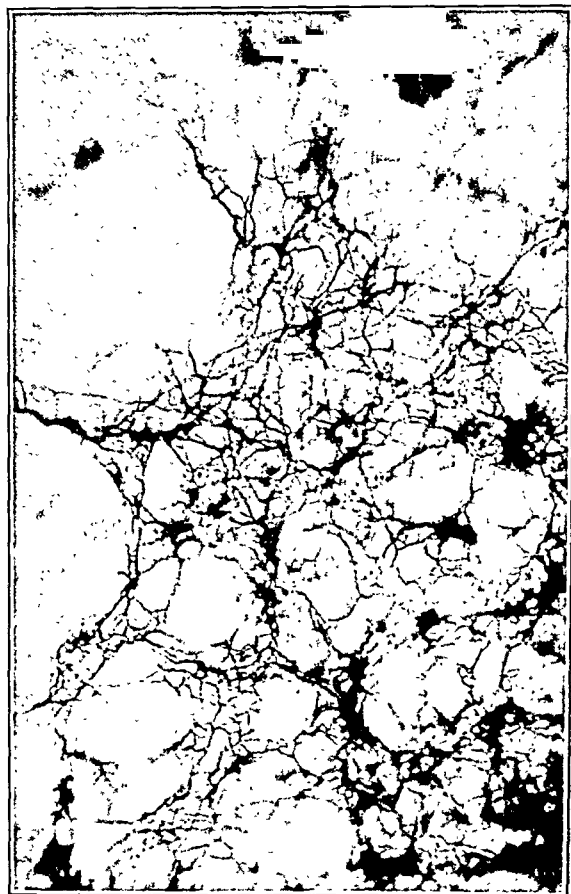
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pear quite prominent. Areas of degeneration or necrosis are never evident to the unaided eye.

The microscopic reaction is well marked. Different phases of it may be simulated and even duplicated in some other severe toxemias, but, on the whole, the reaction is sufficiently characteristic to distinguish yellow fever from other tropical infections with which, at times, it may be confused.

The appearance of the pulp is noteworthy, in so far as it presents two important findings. The first is the absence of an inflammatory reaction. In about 80 per cent of the cases there is a marked congestion of the sinusoids, which brings into unusual prominence the architectural structure of the pulp. Unlike the active splenic hyperemia of septicemia and typhoid, the congestion of the spleen in yellow fever is rarely accompanied by any increase in the white blood elements. One recognizes without much difficulty that the condition is essentially not of the nature of an acute splenitis. Secondly, the fixed tissues of the pulp are not hyperplastic. This is in marked contradistinction to the condition caused by relapsing fever, malaria, and Weil's disease, in which one observes a notable increase in the endothelial framework. As we shall see, proliferative changes in the yellow fever spleen are confined almost entirely to the malpighian corpuscles.

The reticulo-endothelial cells of the pulp frequently show primary enlargement and are somewhat more active than usual in phagocytizing the red blood cells. In most cases the pulp contains large wandering cells of a peculiar type, which we shall presently consider.

The most striking changes are to be observed in the malpighian corpuscles. By examining the spleens of a large number of individuals who died at different stages of the disease, we were able to follow a definite sequence of alterations, the quality of which may be better understood if we first review briefly the normal cytology of the lymphoid follicle.

The observations of Thiel and Downey (1921) show that the malpighian corpuscle has a supporting framework, or reticulum, formed originally by slender strands of vascular endothelium which grow out from the central arteriole and its tiny branches. A lymphogenic function is conceded to the cells of this reticulum, but whether or not all the lymphocytes of the normal follicle are manufactured *in situ*, is in question. The splenic corpuscle often presents a germinal center

## THE PATHOLOGY OF THE SPLEEN IN YELLOW FEVER \*

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In a former communication (Klotz and Simpson, 1927) the pathology of the spleen in thirty-five cases of West African yellow fever was discussed. Since that time we have examined splenic tissues from many more cases, both human and animal, from America and Africa, and these later studies have led to a more comprehensive consideration of the subject.

The pathological diagnosis of yellow fever rests mainly upon the recognition of the specific necrosis (Councilman lesion) in the liver; but not infrequently postmortem changes or poor fixation mask the true nature of the changes in that organ, and render the tissue unsuitable for study. In such cases considerable assistance may be obtained by the microscopic examination of the spleen, for the characteristic alterations in this organ are not so readily obscured after death. Again, it may occasionally happen that the particular block of tissue cut from the liver of a yellow fever victim will show no typical lesion. In such instances, observation of the changes in the spleen, and to some extent in the kidney, will prevent the true diagnosis from being passed over in the interim before further examination of the liver is possible. Aside from having a diagnostic importance, the pathology of the spleen is not without interest, for it illustrates a peculiar effect of the unknown toxin elaborated in yellow fever.

In the gross, the yellow fever spleen is characterized by the absence of distinctive changes. There is no appreciable alteration in size unless the individual has previously been infected with chronic malaria, which is frequent enough, or with some other complicating disease which produces splenomegaly. The organ is usually dark in color, flabby, and at times friable in consistency. On the cut surface the malpighian bodies are, as a rule, reduced in prominence and lacking in definition, but they may, on the other hand, occasionally ap-

\* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

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The sharply defined nuclear membrane and the chromatin are deeply stained and contrast with the pale nucleoplasm."

The nucleus has a gently undulating surface which is characteristic; and not infrequently karyokinetic forms are manifested. Occasionally one or two of these cells may be found in a normal splenic follicle, especially in relation to the germinal center, while in many instances of acute febrile toxemias they are noticeably increased; but in the early splenic reaction to yellow fever they have a quantitative prominence which makes them a distinctive feature. This phase of the reaction is usually superseded, when death occurs, by changes in which the large mononuclear cell plays a less conspicuous part. Only about 20 per cent of our cases presented this early extensive mononucleosis.

Since the cell in question bears little or no resemblance to an endothelial leucocyte and is not phagocytic, the term "free endothelial cell" is probably misleading. It is more correctly designated as a primitive form, probably belonging to the lymphocytic series, and its presence is to be viewed as the result of a peculiar stress brought to bear upon lymphopoietic tissue. It is even more prominent in the lymph glands under identical circumstances. We shall, therefore, refer to this cell as a "primitive mononuclear," for the purpose of clearly differentiating it from the phagocytic large mononuclears of the blood and the cells of the reticulo-endothelial system.

Close upon the initial expansion of the follicle comes a diminution in the number of small lymphocytes. This is a constant feature of the yellow fever spleen. In many instances the primitive mononuclear cells are accentuated by want of the normal constituents of the follicle. The whole organ eventually becomes impoverished of small lymphocytes. The follicles become reduced in size and definition. Sometimes the disappearance of lymphocytes is so marked that many follicles virtually fade out of the picture, leaving only the arteriole and reticular skeleton.

As the small lymphocytes become less and less numerous, the primitive cells likewise gradually diminish, and the endothelial reticulum of the follicles becomes more and more hyperplastic. New cells bud off from the branching structure as from a vine. Not uncommonly one finds the lymphoid elements of the follicle almost completely replaced by a proliferation of small ovoid cells, which at

morphologically comparable to that of the lymph node. Under ordinary circumstances the endothelial framework is so distended with small lymphocytes that it is not recognizable, save in the periphery of the follicle where crowded concentric strands sharply divide the lymphoid elements from the adjoining pulp. The cells of the reticulum are connected only by slender cytoplasmic processes; their nuclei are slightly ovoid and tend to be somewhat vesicular.

Probably the earliest alteration found in the spleen in yellow fever is the appearance of large, unusual, mononuclear cells in and about the follicle. By the addition of these cells the follicle may become somewhat larger than normal. Usually its outline is blurred during this expansion process, but it may, on the contrary, become more sharply defined owing to the resistance of surrounding tissues.

The large mononuclear cells met with under these conditions are usually prominent, and often show a striking deviation from the normal. They are scattered through the lymphocytes, usually most numerous about the periphery of the corpuscle, where they sometimes form a mantle encircling the whole structure. In sparser fashion they are scattered over the pulp areas. They bear no regular relation to the fixed tissues and appear to migrate freely from one part of the spleen to another.

It is unusual to find any trace of germinal centers during this phase of the reaction. The large mononuclear cells dominate the whole follicle. These cells are somewhat variable in appearance but apparently belong to one type. They probably have their origin *in situ*. The structure of the nucleus varies from that which is typical of the majority, to that of large semidetached reticular cells, and it is not difficult to trace a transition between the two. Some of the intermediate forms are large, oddly shaped, and vesicular, but the majority are fairly uniform.

Turnbull (1913) described them thus: "The Malpighian bodies contain, in addition to lymphocytes, an increased number of cells which, in default of any generally accepted name, will be referred to here as 'free endothelial cells.' These cells are approximately round and occupy an area equal to from three to four-and-a-half red corpuscles. The protoplasm is non-granular and basophil. The nucleus occupies half or more of the cell. The chromatin is arranged as a, usually wide meshed, net of narrow rods, and is also massed as a stout capsule round each of the three or four large nucleoli.

scribed above, appear in the spleen in acute febrile states other than yellow fever, but they are rarely found in cases of chemical poisoning. They are to be interpreted as a response to a severe toxemia.

Primary enlargement of the newly developed reticular cells is observed around the periphery of the follicle. The nuclei are swollen and vesicular, presenting many bizarre forms which probably represent preliminary steps in the genesis of the primitive mononuclear cells.

About 20 per cent of our preparations show the presence of a few large multinucleated giant cells scattered through the pulp. They resemble the megakaryocytes of the bone marrow. Turnbull believed them to be derivatives of the large mononuclear cells. They are seldom quantitatively prominent, and they occur in the splenic reaction of other conditions. Indeed, it is not uncommon to find an occasional giant cell in the relatively normal spleen.

In those spleens which may be said to be in the end stages of the yellow fever reaction, one finds many curious masses of nuclear débris lying in the pulp. The fragments making up such a mass are usually in the shape of rings and crescents, and are jumbled together, forming a large, composite, irregular nuclear body without any apparent cytoplasm. The phenomenon may represent the phagocytosis of a number of small degenerate nuclei by a large pulp cell which has itself degenerated, or it may be simply a retrograde change in a multinucleate giant cell. We have not observed such masses in conditions other than yellow fever.

A moderate eosinophilia is often present in the spleen of advanced cases. The granulocytes are of a mature type and appear to be scattered through the pulp. There is no evidence of myelogenous proliferation within the spleen.

When the walls of the malpighian arteriole become severely damaged it is not uncommon to find hemorrhage into the follicle. Such a lesion is, however, of no moment.

#### DISCUSSION

In a limited number of cases, we have had opportunity to compare the changes of the splenic corpuscles with those of lymph glands in the same individual, and we have found a striking similarity. These synchronous changes in the main lymphoid depots of the body lead to the conclusion that the changes in the splenic corpuscles are

first sight appear to be degenerated lymphocytes, but on closer examination prove to be reticular cells, joined by frail cytoplasmic processes and growing in a compact mass. One can usually make out a few small lymphocytes and a few primitive mononuclear cells in the meshes of this endothelial hyperplasia.

With this activity on the part of the fixed tissues of the follicles, germinal centers spring into existence and frequently become prominent. These foci of growth often arise in relation to a small arterial twig, the wall of which appears to supply the parent tissue. Such germinal centers lack the loose cellular structure of the normal and are prone to degenerative changes.

When the endothelial hyperplasia reaches these proportions, degeneration invariably overtakes it. Indeed, at this stage the whole organ shows an intense toxemia, and has a peculiar appearance. Nearly all nuclei throughout the whole tissue tend to be somewhat washed-out or vesicular, and the cytoplasm is hyalinized and often vacuolated.

Many of the primitive mononuclear cells in pulp and follicle appear to be undergoing mitosis, but the chromatin material lacks the delicate structure of normal karyokinesis. With coincident evidence of retrograde changes in the cytoplasm, the mitotic process is to be viewed as a perverted response of a degenerating cell rather than as normal cell division.

Degenerative changes are most prominently featured in the malpighian corpuscles. The wall of the central arteriole is transformed into a pale homogeneous hyaline substance which spreads out as if it were in a plastic state. As a result the contour of the vessel becomes distorted. The tiny branches of the main arteriole are similarly affected.

The peculiar germinal center found in this phase of the reaction undergoes severe retrograde changes during the process of growth. It appears to be a fused mass of pale waxy cytoplasm, similar in consistency to the substance of the arteriolar wall. Often it contains nuclear débris and sometimes is the site of necrosis attracting polynuclear leucocytes. The mass is occasionally sharply limited by the surrounding tissues, as if it had developed by a rapid, expansive growth.

False germinal centers, as we term the abortive formations de-

Changes in lymph glands parallel those of the splenic corpuscles, which is evidence of the fact that yellow fever toxin has a selective action upon lymphopoietic tissue.

We have found a careful examination of the spleen to be frequently helpful in facilitating a pathological diagnosis where yellow fever must be differentiated from other conditions giving rise to liver and kidney damage.

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### DESCRIPTION OF PLATES

#### PLATE 124

- FIG. 1. Normal splenic corpuscle showing part of germinal center. From an individual who died instantaneously with a fractured spine.  $\times 200$ .
- FIG. 2. Early reaction in splenic corpuscle in yellow fever. A marked mononucleosis may be seen.  $\times 200$ .

but a part of the general reaction of the whole lymphopoietic system. The leucopenia associated with yellow fever is probably a related effect of the yellow fever toxin.

Toxins of different diseases frequently manifest some selective action on different parts of the hematopoietic system. Thus, many produce an anemia, others a polynuclear leucocytosis, and still others an abnormal activity on the part of reticulo-endothelial cells. None of these effects are observed in yellow fever, but instead there is a leucopenia and evidence of a progressive irritation and cytolysis in the lymphogenic tissues.

In the spleen there is an absence of the lesions which characterize the liver pathology of the disease. The Councilman necrosis is lacking, fatty change is negligible, and the nuclei present no suggestion of specific inclusion bodies.

#### SUMMARY

In yellow fever, the spleen, like the liver, presents no distinctive gross features comparable to those seen under the microscope.

Active hyperemia is met with in about 80 per cent of cases, but is unaccompanied by a leucocytic infiltration.

There is absence of hyperplasia in the fixed tissues of the pulp.

Changes in the malpighian corpuscles characterize the splenic picture of the disease. Here, we recognize four phases of the reaction:

1. *Mononucleosis*. The type cell is an undifferentiated mononuclear derived from the reticular tissue of the follicle; it never entirely disappears during the entire course of the disease.

2. *Lymphopenia*. There is a striking loss of lymphocytes from the whole organ which persists throughout the reaction.

3. *Hyperplasia of the fixed tissues of the follicle*. False germinal centers are formed.

4. *Degeneration*. This is manifested throughout the whole spleen by vesicular nuclei and waxy degeneration of cytoplasm. False germinal centers undergo retrograde changes, amounting sometimes to actual necrosis. Pseudomitosis of the primitive mononuclears is observed. Large fragmented nuclear forms appear in the pulp.

A third of the cases show a few large multinucleate giant cells resembling megakaryocytes.

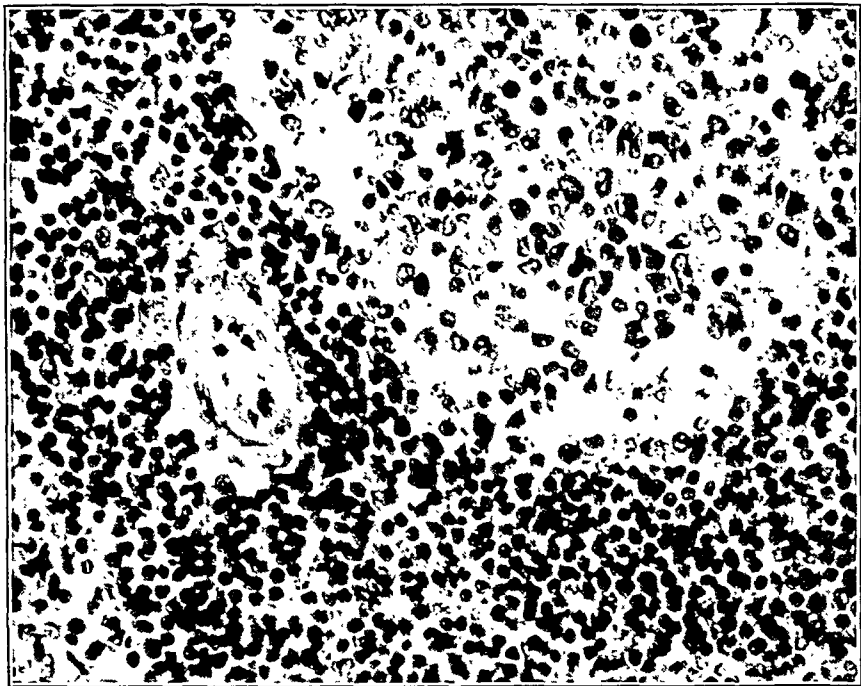
A moderate eosinophilia is commonly observed in the later stages of the reaction.



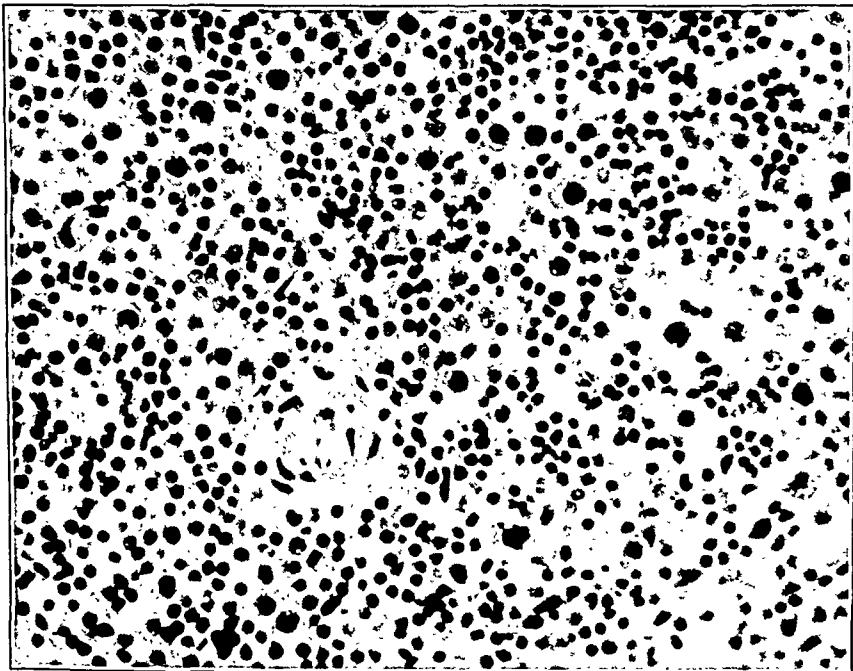
PLATE 125

FIG. 3. Second stage of yellow fever reaction in splenic corpuscle. Loss of lymphocytes is well marked. The reticulum of the corpuscle is prominent and large mononuclears may be seen budding from it.  $\times 200$ .

FIG. 4. Later stage of yellow fever reaction in splenic corpuscle. The wall of the arteriole is swollen and hyalinized. The reticulum is hyperplastic and degenerate. On the left is part of degenerate false germinal center. There is almost a complete absence of lymphocytes.  $\times 200$ .

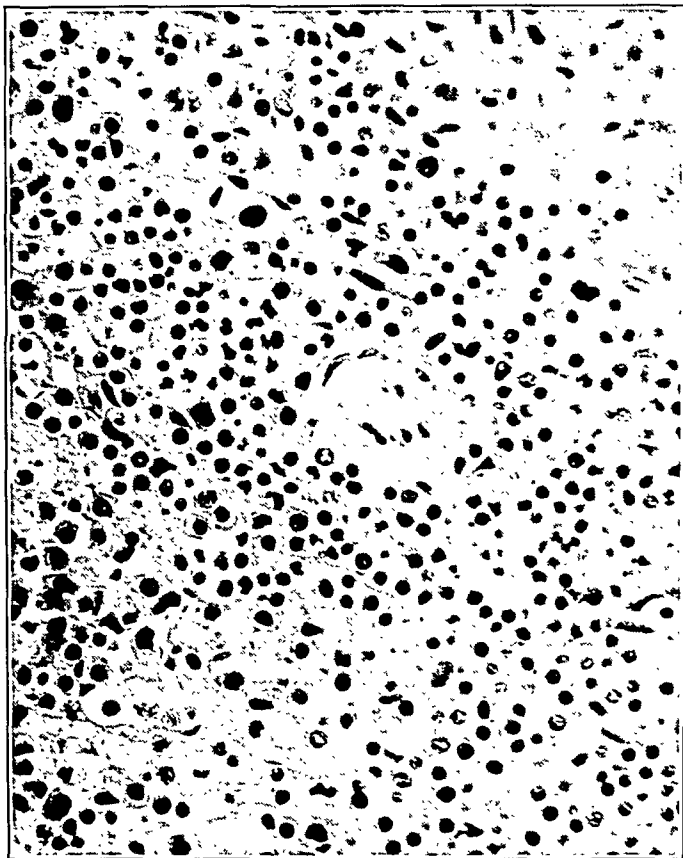


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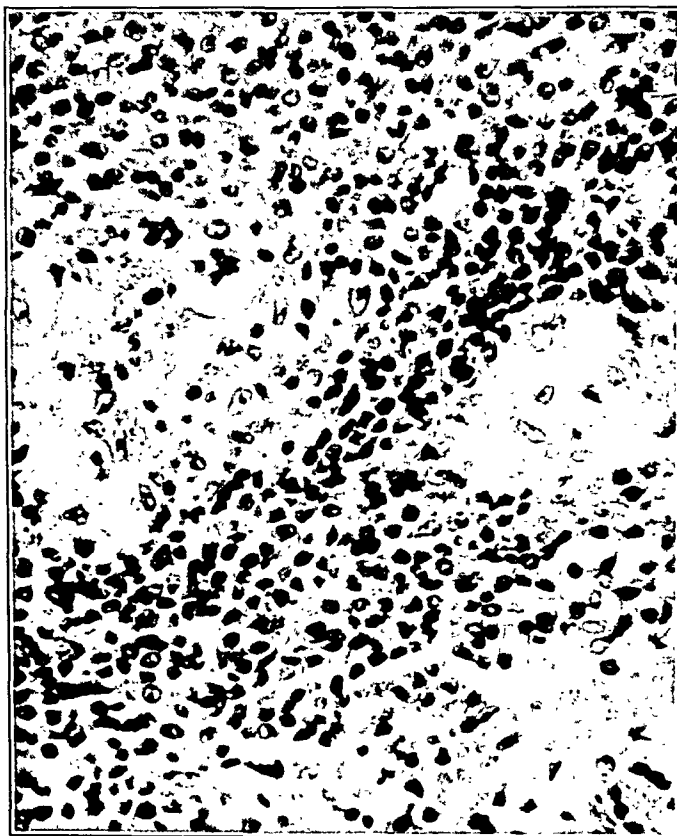


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carried out at the laboratories of the West African Yellow Fever Commission of the Rockefeller Foundation at Yaba, Nigeria, is included in the group. Care was taken to select only tissues obtained immediately after death and in which fixation had been properly carried out.

Several methods of fixation were used: Zenker, formalin, and Müller-formol. For general purposes of cytoplasmic and nuclear study the Zenker fluid without acetic acid served best. Formalin-fixed tissues of each case were cut on the freezing microtome and stained for fat with sudan-scharlach R and Nile-blue sulphate. Besides the routine hematoxylin-eosin stain, a number of other staining methods were applied to the Zenker paraffin sections, such as special Giemsa, phloxine-azure-B-bromide for intranuclear changes, Perle's stain for iron, Best's carmine for glycogen, Van Gieson's for stroma reticulum, and Goodpasture's fuchsin. We found it essential that the paraffin sections should not be more than four microns in thickness, otherwise the minute cytoplasmic and nuclear changes tend to be masked.

For comparison, forty-five cases of liver necrosis resulting from causes other than yellow fever were studied. This group included cases of acute yellow atrophy, eclampsia, Weil's disease, typhoid, relapsing fever, and six different kinds of poisoning.

In another paper we discuss the identity of the pathological lesions in the yellow fever cases occurring in Africa and in America. There is no longer need to designate the diseases upon these two continents by separate names, such as African yellow fever and American yellow fever. It is now an established fact that there is but one yellow fever, and the findings reported in this paper apply to the disease in both hemispheres.

### GROSS PATHOLOGY

Macroscopic examination of the liver of the yellow fever victim does not reveal constant and characteristic changes such as are seen under the microscope. It is not often that the amount of damage visible to the unaided eye arrests the attention in so striking a manner as does that which is shown by the microscope; and in no instance is it possible to make an accurate estimate of the extent of the liver necrosis by examination of the gross specimen alone. In this respect

## THE PATHOLOGY OF THE LIVER IN YELLOW FEVER \*

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Renewed interest has developed in the study of yellow fever since the reports of the West African Yellow Fever Commission of the Rockefeller Foundation, through whose efforts, in 1926, it was shown that the *Leptospira icteroides* is not related to the disease in West Africa, and that the guinea pig is not susceptible to the disease. Until 1927 yellow fever had never been experimentally produced in the lower animals. In that year the Commission undertook the task of finding a susceptible animal and was finally successful with the *Macacus rhesus*. Reproduction of the disease in this animal has facilitated research and has given pathologists an opportunity to learn something of the nature of the yellow fever virus. Although, up to the present, no visible organism has been discovered, it is known that the infecting agent possesses the qualities of a filtrable virus, both in its behavior under extraneous influences and in its attack upon susceptible tissues.

With the knowledge that yellow fever belongs to the group of virus diseases, we have given particular attention to the examination of the liver for the purpose of determining the nature of the specific changes which this organ undergoes during an attack of the disease. The importance of definite knowledge concerning cytoplasmic and nuclear changes is accentuated by the fact that not a few of the virus diseases show peculiar, and at times specific, intracellular changes by which they may be identified. The present study is confined to the liver, for the reason that no other organ or tissue has been found to show such constant characteristic lesions.

Our series of cases represents, without exception, natural fatalities from yellow fever. Human material was obtained from fifty West African cases and forty-three American cases. In addition, material from nineteen *Macacus rhesus*, made available through researches

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nent feature. Jaundice of the liver and other structures of the body is most pronounced in individuals dying after the eighth or ninth day of illness. Ordinarily the size of the liver remains normal, or is slightly increased. The enlargement is never great and is caused by a slight diffuse edema involving both the stroma and parenchymatous cells.

On section there is evidence of cloudy swelling. The cut edges of the organ project upward and are everted. The larger portal vessels are sunk below the plane of the cut surface. The fresh tissue is a definite pinkish gray-yellow, a dull indefinite color, which becomes lighter and yellower as it remains exposed. The lobules are indistinct and cannot be clearly defined; frequently the central vein cannot be recognized. This lack of distinctness is no doubt due to the swelling of the lobules and the compression of many of the vascular channels, including, to some extent, the central vein. Some investigators have intimated that in the early stages of the disease the central vein area of the lobule is greatly congested, while in later stages a more uniform appearance of the cut surface is encountered. These differences in appearance can probably be accounted for by the differences in time after death at which the autopsy was performed. With the drainage of the blood from the liver, the structure of the organ becomes more uniform in appearance. It is true in the case of both humans and monkeys that when autopsies are performed immediately after death, the central vein is not uncommonly found to be prominent, as if in a state of passive congestion. This central congestion is, however, never so prominent or so persistent as the passive congestion caused by heart disease. We have never seen definite hemorrhagic necroses in the gross specimen of the liver.

The yellow color which makes its appearance diffusely in the liver and which may at times be described as clay color, at times as box-wood or ochre yellow, does not depend solely upon the presence of bile pigments, but also upon the fatty changes which always, to some extent, accompany this disease. Moreover, it is probable that the small quantity of bile which escapes during the acute disease is absorbed by the fatty materials and gives rise to the peculiar yellow color. As the quantity of fat present in the yellow fever liver varies tremendously, the associated changes likewise vary. The oft-repeated statement that the knife used in sectioning the liver appears greasy, and that quantities of fat may be scraped from the cut sur-

the lesion encountered in yellow fever differs from that associated in our experience with acute yellow atrophy of the liver, and with toxic necrosis of the liver as seen in arsenical, carbon tetrachloride, and other hepatic poisonings. Moreover, since the liver lesion in yellow fever is more uniformly diffuse, except in rare instances in which the left lobe may be less involved than the right, the blotchy mottling observed in other conditions is absent. On the whole, the gross appearance of the liver, the organ most severely involved in yellow fever, is disappointing. It is necessary to make a careful microscopic study of the organ in order to recognize the characteristic lesions.

Since yellow fever is a disease largely confined to hot climates, postmortem changes in the body soon alter the *intra vitam* appearances. Many of the variations in the appearance of the liver which have been described were the result of postmortem processes. A difference of four to six hours in the length of time elapsing between death and autopsy will lead to quite marked variations in the color and external appearance of the liver. Furthermore, the boxwood color of this organ, which has so frequently been mentioned in the literature, makes its appearance only when the blood has been naturally drained away from the organ or has been pressed out after its removal.

The liver maintains its normal contour. It has a smooth and glistening surface, and there is no evidence of peritoneal change. Occasional small petechial hemorrhages may be observed under the capsule, but these are never numerous or large. In the fresh cadaver, not over three hours after death, the liver is of a reddish gray color shading into yellowish gray. At this time it is never yellow. However, if the autopsy is performed six to twelve hours after death, the liver is more decidedly yellow, but still has a reddish cast in its tissues. If the blood is allowed to drain from the liver after the organ is removed from the body, or if a portion of the liver is compressed between the fingers, the exsanguinated portions will appear quite yellow, sometimes even an ochre yellow, the color of boxwood. Only when postmortem changes have progressed, with their secondary tissue damage, have we found the liver of truly jaundiced appearance. At times the liver may appear quite red on its outer surface, with no evidence of extensive necrotic processes in the lobules. This is particularly true of fulminant cases, in which jaundice is not a promi-



shall presently consider in turn, are to be viewed as independent effects of the blood-borne damaging agent of the disease.

There is a wide variation in the intensity of liver injury, but in every instance well marked and characteristic changes are found. The severity of the lesion bears no fixed relationship to the clinical severity of the attack. Some very acute and rapidly fatal cases show only a minimal liver lesion.

### CYTOPLASMIC CHANGES

*Fatty Deposits:* The occurrence of fat in the liver cells, though somewhat variable in amount and distribution, is constantly observed and must be considered as characteristic of the disease. It is laid down in large and small droplets: the former occupy the better preserved cells, the latter the more degenerate cells. Large droplets were met with in only about one-half of our cases, but the finely divided or granular fat was always present. Both forms stain alike and probably represent expressions of the same process, whether it be an accumulation of physiological fat due to a local failure of fat metabolism, as Mallory believes, or a lipoid deposition in the cytoplasm resulting from disintegration of compound bodies. It is not possible to distinguish between fatty infiltration and fatty degeneration in yellow fever livers. The most one can say is that the larger fat droplets are found in the necrobiotic and partly injured cells, which, in the matter of functional activity, stand midway between the apparently healthy peripheral cells and the necrotic cells. Necrotic cells contain only the finely divided fat which may appear in considerable quantity.

The total quantity of demonstrable fat in the liver lobules varies from case to case, and we have never been able to correlate the extent of the fatty change with any particular character of the disease. At times, in fulminant cases, heavy deposits of fat appear in the liver, but at other times a similar condition is discovered in less intense cases where death was caused by renal complications. It must be remembered that extremely fatty livers in which the tissue is loaded with a fat deposit sufficient to catch the eye at autopsy and lead to the comment of "greasy liver," are unusual. When such a liver is found, the fat appears in large globules and occupies the cells in all zones of the lobule.

face, has been overstressed and exaggerated. Some livers are decidedly fatty, and in such cases a block of the tissue will float on water, but this is by no means a common finding.

Only the most careful examination with the magnifying glass will reveal evidence of necrosis. As the necrotic areas are not accompanied by interstitial hemorrhages, and as the fatty-icteric hue pervades the tissue diffusely, there are but few marks by which one may recognize the necrotic areas. Furthermore, this form of necrosis differs from that found in acute yellow atrophy, in that the necrotic liver cells do not undergo rapid dissolution; there is therefore relatively little actual loss of substance at the time of death, on the fifth or sixth day of illness. Hence, also, there is no evidence of the "pitting" or depressions which usually mark the areas of necrosis in the liver in other diseases. There are no thrombi in the portal system, and no change can be distinguished either in the hepatic artery or in the veins. The bile ducts also show no change, although the bile along their tracts and in the gall bladder may be quite viscid and thick.

### MICROSCOPIC PATHOLOGY

Microscopic examination reveals a severity of liver injury which is in striking contrast to the small amount of damage visible in the gross. In the preliminary survey of the tissue a marked disorganization of the parenchyma is at once apparent. There is jumbling of the liver cords to a greater or lesser degree, with proportionate distortion of the sinusoids. The injury consists in a process of necrosis and necrobiosis which is remarkable in several respects; the entire lesion is of a non-inflammatory character and lacks an exudative response save in the presence of a secondary process; it is more diffuse than focal in character, but in the majority of cases involves chiefly the midzones, tending to spare the peripheral and central parts of the lobules; evidence of a rapid autolysis is lacking so that there is no alteration in contour nor reduction in size of the lobules, such as one might expect in consideration of a widespread cell destruction; the structures of the portal sheaths are practically unaffected, and interstitial hemorrhage is rarely found.

A peculiar form of necrosis invariably dominates the microscopic picture, but fatty change and cloudy swelling contribute to the lesion in every case. These three degenerative processes, which we

man or animal. The necrosis may apparently supervene at any stage of the degenerative process, though it is probably preceded in all cases by some degree of fatty and cloudy change.

The proportion of the lobule actually necrosed varied roughly from 5 to 100 per cent in the different individuals of our series, with the mean average somewhere about 80 per cent (see Chart I). An appreciable number of the human cases showed comparatively slight damage to the liver, having less than a tenth of the hepatic cells necrotic. It is in cases of slight damage, such as these, that the

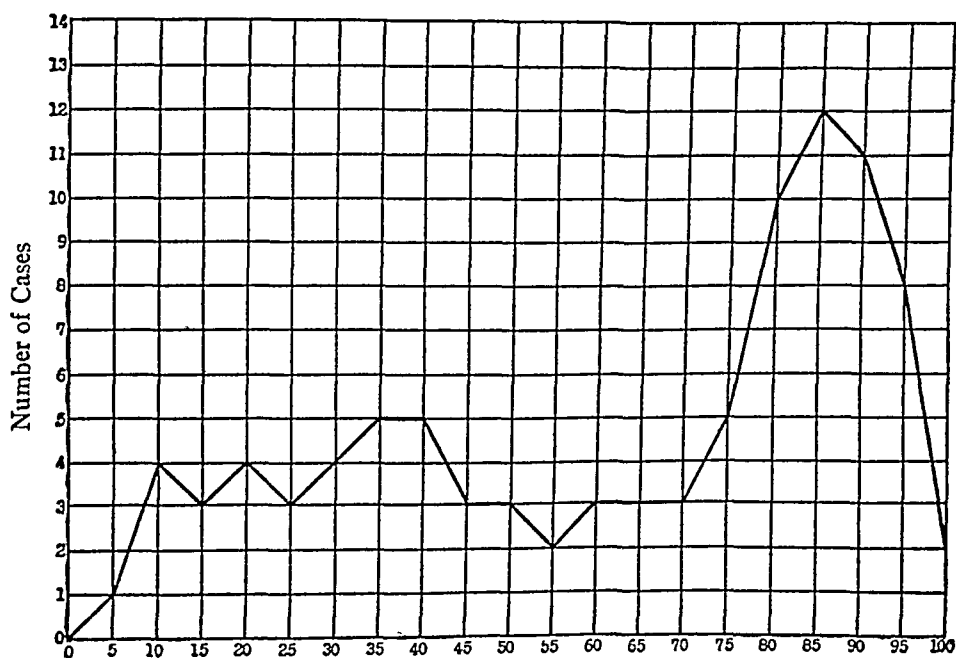


CHART I

Percentage of Average Lobule Destroyed by Necrosis

peculiar distribution of the necrosis is best demonstrated. It runs to all parts of the lobule and is variously described as diffusely sprinkled, scattered, sporadic, or patchy. The cells of a given liver column are affected discontinuously; single cells at irregular intervals are attacked in part or as a whole, or small groups of two or three adjacent cells fall prey together.

As greater proportions of liver cells become necrosed, midzonal destruction is more and more emphasized. This localization, stressed by Rocha Lima, is well marked in livers which have between 30 and 90 per cent of the average lobule involved in necrosis. Midzonal

The zonal distribution of fat is often considerable, and shows itself chiefly in relation to the midportion of the lobule. This zone, even when showing a well marked zonal necrosis, contains a sufficient number of necrobiotic cells to make the deposit prominent. However, in other cases where the midzone necrosis is well advanced, a fatty zone may delimit the necrotic area on each side so that an apparent peripheral as well as central zone of fatty degeneration is found. Moreover, the fat may appear in unequal quantities and distribution in the different lobules of the liver.

When stained with Nile-blue sulphate, some of the granules in the necrotic cells manifest the reaction of fatty acids or their compounds, but throughout the lobule the droplets are almost entirely of the nature of neutral fat.

*Cloudy Swelling:* Some degree of cloudy swelling is constantly present in the liver parenchyma. We have found scarcely a single instance of a normal liver cell in all our preparations. This type of degeneration is manifested chiefly by a finely granular appearance of the cytoplasm and a moderate edema in the better preserved cells. It is a diffuse and even change of the cytoplasm involving little alteration in staining qualities. The granules are only obscured when the cytoplasm undergoes coagulative hyalinization. When the necrotic process occupies chiefly the midzone of the lobule, the cells in both the peripheral and central zones show cloudy swelling. The swollen appearance of many of the cells is, of course, accentuated by their engorgement with fat. It is probably owing to this edema and fat deposit that the liver cells are larger than normal, accounting for the slightly increased bulk of the liver despite the presence of parenchymatous necrosis.

*Hyaline Necrosis (Councilman Lesion):* The degenerative process involving the liver lobule may develop into an advanced stage of necrobiosis with the manifestation only of fatty change and cloudy swelling. The cytoplasm of the cell under such circumstances is markedly rarefied and granular, while the nucleus is swollen and hydropic. But it has not yet passed the point where recovery is impossible. It is only with the manifestation of hyaline necrosis, referred to elsewhere as the Councilman lesion, that the cells disintegrate and death occurs. This peculiar change, more than any other, characterizes the liver lesion in yellow fever. In our experience it is present in all cases of natural fatality from the disease, whether hu-

are seen to contain granular fat. Very often remnants of nuclei and granules of yellowish bile pigment are seen within the hyaline mass.

The cells or parts of cells undergoing necrosis invariably become rounded into spherical forms. This distinctive morphological feature adds to the prominence of the hyaline bodies. These bodies tend to shrink in the process of preparation so that they are often emphasized in outline by a narrow clear zone, artificially produced. In many specimens such discrete bodies are quite numerous and at one time they were thought to be amebae or some other unicellular parasite — an interpretation which their morphology does, indeed, suggest, although their variation in size discounts it. They range roughly from five to twenty-five microns in diameter.

Frequently an elongated nucleus is observed, flattened against the outline of the necrosing cell in such a way as to simulate the contour of a signet ring. Rocha Lima interprets these nuclei as belonging to wandering cells which have penetrated the necrotic mass of cytoplasm; but whatever their origin, signet ring forms are often quantitatively and morphologically prominent in the parenchyma of the yellow fever liver.

The spherical hyaline bodies soon lose their contours and disintegrate. This process is hastened by postmortem change, poor fixation of the tissue, and advanced stages of necrosis. Thus, in many instances, the identification of the Councilman change rests more upon the staining reaction and the consistency of the affected cells and cell parts than upon their morphology. In cases where the liver damage is maximal, whole lobules may be virtually littered with amorphous fragments of necrotic cytoplasm, which, however, retain their distinctive hyaline character.

The technical process of preparing microscopic sections probably exaggerates the natural tendency to crumble which the hyaline masses possess. Portions of necrosed cells often appear to lie free in the sinusoids. This phenomenon led Councilman to speculate as to whether emboli might not thus arise. It seems probable, however, that such fragments are detached by the microtome knife, and are not free within the blood channels during life.

In many instances fragments of hyalinized cytoplasm closely resemble distorted red blood cells, and on casual observation, the two might easily be confused. Not a few authors have insisted upon the frequency of interstitial hemorrhage in the yellow fever liver, but we

areas of concentrated destruction are, however, in the majority of cases, ill-defined and shade off peripherally and centrally into the sporadic lesion which reaches to the outer and inner limits of the lobule. A narrow zone of cells surrounding the portal sheath is the last part of the lobule to be destroyed; its preservation is another of the characteristic features of the yellow fever liver.

In most cases both the sporadic and zonal distribution of necrosis may be identified, but the former is constant and specific, while the latter is uncertain as a diagnostic criterion. The smaller the proportion of necrosed cells in a lobule, the more scattered or sporadic is the lesion, and the less definite the midzonal concentration. Conversely, when the midzonal emphasis is lost because of complete destruction of the lobule, the sporadic selectivity may still be distinguished in the varying intensity of necrosis from cell to cell.

Not all parts of the liver are affected to a like degree. It is usual to find the lobules in any one microscopic section more or less uniformly involved, but in a few instances exceptions to this rule are encountered. Lobules lying side by side may be affected in a very unequal fashion. In two cases blocks of tissue cut from the left lobe of the liver failed to show lesions characteristic of yellow fever, while blocks from the right lobe, subsequently examined, gave a typical hepatic picture of the disease.

Necrosis of the parenchymatous cells, whether scattered sporadically or arranged more or less in zones, is always readily recognized by the peculiar, acidophilic, hyaline change of the cytoplasm, which has no counterpart in other diseases. Scattered focal necroses have been described in a number of diseases, and midzonal necroses may arise in severe sepsis and peritonitis, but in none of these do we find the characteristics of cell necrosis as we find it in yellow fever.

Ordinarily the parenchymal cytoplasm is neutrophilic or faintly basophilic, according to the degree of differentiation. The necrosed cells and parts of cells have, by comparison, a marked affinity for eosin and like dyes, which makes for sharp contrast with the surrounding cells (Fig. 1).

In consistency the necrosed parts are denser and more refractile than normal cytoplasm. Their substance is of a homogeneous character, which, coupled with acidophilic properties, has given rise to the designation "hyaline necrosis" or "hyaline bodies." They are, as a rule, honeycombed with small vacuoles, which in frozen sections

that in the latter there are many changes which may well be attributed to the technique of dehydration. In the former, the nuclear membrane is well filled with a watery nucleoplasm in which small, dull, chromatin granules are scattered more or less evenly. The nucleoli can easily be identified but they are not prominent. In paraffin sections, on the other hand, the same nuclei usually present a "washed-out" appearance. Much of the chromatin appears to have been lost, while the remainder is granular and collected in small irregular clusters on the inner surface of the nuclear membrane and the outer surface of the nucleolus. The remaining intranuclear space is optically clear. The chromatin shows a loss of affinity for the basic dyes and varies in color from bluish purple to dull red. In this type of nuclear alteration there is no apparent cleavage between the basophilic and acidophilic elements of the chromatin, but the whole undergoes a more or less uniform change. This is the important point of distinction between the non-specific nuclear change, and the so-called inclusion phenomenon in which a definite separation of blue-staining and red-staining chromatin appears to take place. As a rule, the nucleoli stand out very prominently; occasionally they take on a faint blue tint, but for the most part they are stained reddish pink. There is a striking frequency of two and three nucleoli within a single nucleus. Where multiple nucleoli are not seen, there is often marked increase in the size of the single nucleolus.

A second type of nuclear change is much less prominent, but is more typical of yellow fever in a qualitative way. We refer to the so-called "inclusion bodies" and alterations associated with them. The term "nuclear inclusion bodies" has come to have a definite meaning which one is at a loss to convey tersely except by some such arbitrary designation. It is misleading in the sense that it implies the presence of extraneous matter within the nucleus. This conception is by no means justified; the term is applied to bodies whose nature and mode of development are comparatively unknown; therefore, it does not lend itself to accurate understanding unless used by the author in a specific and restricted sense.

The nuclear inclusions first described by Lipshütz as peculiar to lesions produced by herpes virus are of a definite character, both quantitatively and qualitatively. They occur abundantly in experimental lesions and are easily demonstrated by the ordinary hematoxylin-eosin method. For these reasons herpetic inclusions have

believe that this erroneous interpretation has arisen through failure to make a critical distinction between erythrocytes and necrotic fragments of cytoplasm. The former are never vacuolated, as are the latter.

The nature of the distinctive hyaline change is not clear, except that it represents a coagulative, rather than an autolytic process. There occurs a condensation of the cytoplasmic substance in which may remain incorporated some of the fat and pigment contained in the cell before its necrosis. The development of the discrete hyaline spherules within the membrane of the living cell probably represents a localized coagulative change, wherein a diffusely hyalinized patch of cytoplasm becomes isolated and assumes a globular shape.

In only one of the forty-five cases of liver necrosis in affections other than yellow fever, which were studied for purposes of comparison, did we find a condition resembling the peculiar pathology of yellow fever. This was a case of cellulitis and pneumonia in an infant, presenting scattered necrotic cells in the liver parenchyma. These cells were completely hyalinized and did not show the partial hyaline coagulation of cytoplasm so often observed in yellow fever. The necrosis had changed the staining properties of the affected cells from neutrophilic to acidophilic, but failed to give rise to that homogeneous change which serves to accentuate the hyaline masses in yellow fever. Fine fatty vacuoles were also absent from the hyalinized cytoplasm, which constituted a further difference; and the distribution of the necrotic cells was less diffuse and more focal in nature than in yellow fever.

### NUCLEAR CHANGES

*In the Human Liver:* Nuclei of the liver cells in yellow fever react in a variety of ways, many of which are not distinctive of the disease. The commonest change is a combination of nuclear edema, chromatolysis, and acidophilia of the intranuclear constituents, and although this combination is found in nearly all yellow fever livers, it also occurs frequently in other conditions.

These changes are confined almost entirely to the cells of more normal appearance which are affected only with cloudy swelling and fatty vacuolation. The intranuclear appearance differs considerably in frozen and in paraffin sections. A comparison of the two shows



in further contrast to the swollen nuclei previously described, are confined almost entirely to necrobiotic or actually necrotic cells. The nuclear membrane stands out sharply as if outlined in India ink. It is invariably finely beaded with small dark droplets and granules of chromatin. Not infrequently the nuclear outline is distorted in shape. The end-stage inclusion fills the whole intranuclear space with a dull, pink, homogeneous ground substance. Such an inclusion has lost its distinctive character, and cannot be accurately spoken of as a specific change, for several of the control livers present similar forms.

Of the whole series, then, twenty-three were positive, twenty-seven doubtful, and forty-three negative for intranuclear inclusion bodies.

In view of the comparatively small proportion of cases manifesting the phenomenon, we investigated the series carefully to ascertain whether any factor could be correlated with the presence or absence of inclusions. We found that age, sex, and race have apparently no bearing on the matter. The length of time intervening between death and autopsy was equally variable, and within the usual limits in both positive and negative cases. Cases with Zenker-fixed tissue show a slightly higher incidence of inclusion bodies than those with formalin-fixed material. However, the method of preservation seems to be less important than the length of time the tissues have been kept. The incidence of inclusions was four times as high in the African as in the American cases of our series, but this was probably due in part to the prolonged formalin fixation to which the bulk of the American tissues had been subjected. From the examination of different groups of cases which had occurred in different epidemics, it was suggested that some strains of virus might perhaps exceed others in their ability to produce specific nuclear changes; but if one factor stands out more than another in relation to the presence of inclusion bodies, it is the duration of the illness. Among the positive cases, none had been ill longer than 6 days, and the average duration was 3.8 days. The negative cases ranged between 3 and 12 days of illness, averaging 5.8 days. These observations bear out Torres' suggestion that inclusions are present only during the period when the virus is free in the blood stream. There remains to be explained, however, the absence of inclusions in certain of our cases that died in the early (infective) period of yellow fever, and the conclusion pre-

been studied more extensively than other types, and have come to be looked upon as the standard example. From a study of herpetic inclusions in all stages of their development, supplemented by a review of the literature, the following broad definition suggests itself as a criterion by which we may judge the presence or absence of the phenomenon in yellow fever livers: An intranuclear inclusion body is (1) not a preformed part of the nucleus, as for instance, chromatin or nucleolus; (2) markedly acidophilic, so that it contrasts with other parts of the nucleus when stained by ordinary methods; (3) associated with preservation of the nuclear membrane and migration of basophilic chromatin to the periphery of the nucleus; (4) surrounded or partially surrounded by a clear space; (5) not possessed of a constant size or morphology; (6) single or multiple in a single nucleus; (7) made up of fine granules, which are more or less uniform in size; (8) formed of an unknown substance which has been shown by Cowdry (1928) to possess certain constant microchemical properties; (9) not of the nature of an artefact since Goodpasture and Teague (1923), and Cowdry and Kitchen (1929, 1930) have demonstrated it in the fresh unstained cell.

Cowdry and Kitchen (1930) have completed a much more exhaustive study of nuclear inclusions in human yellow fever than opportunity has permitted us to undertake. These authors have contributed a comparative description of the known types of specific nuclear bodies as they occur in fresh, frozen, and fixed tissues, including microchemical properties and staining reactions to various dyes. The authors direct special attention to the peculiarities of the inclusion phenomenon as it occurs in yellow fever; they were able to demonstrate specific nuclear bodies in ten out of thirty-nine human cases.

We observed definite nuclear inclusion bodies in twenty-three of ninety-three human cases. In each of these the typical bodies described by Cowdry and Kitchen were found, often in such numbers as to form a striking feature of the histological picture. Twenty-seven additional cases presented a nuclear change which we interpreted as being closely related to the formation of inclusions. Indeed, they conformed to the picture which Cowdry and Kitchen describe as the end-stage. This atypical form was seen also in the twenty-three cases positive for typical inclusions.

The nuclei containing the inclusions are, as a rule, quite small and,

The associated retrograde changes in cytoplasm also argue against normal mitosis. All the evidence, on the contrary, points to a degenerative change in the nucleus in which the metaphase and less often the anaphase of mitosis is simulated. We are of the opinion that active liver regeneration is at a low ebb during the acute stages of the disease and that few true mitoses are found in any part of the lobule. In reviewing the cases it was surprising to note that karyokinetic figures were no more frequent in individuals surviving the tenth to twelfth day of illness, in whom liver necroses were still the outstanding lesion, than in those who had died as early as the third day.

*In the Macacus Rhesus Liver:* The nuclear inclusion phenomenon as it relates to yellow fever, was first observed in the livers of experimental animals and described by Torres in 1928. Subsequently, as we have seen, the phenomenon was identified in the human liver. The incidence and character of intranuclear inclusions in the monkey are, however, quite different from those in man. Cowdry and Kitchen (1930) have dwelt fully upon this interesting feature and have advanced some plausible suggestions as to the cause of the discrepancy, but an adequate explanation is lacking.

Of our total nineteen *M. rhesus* livers, seventeen were positive for typical inclusion bodies, one was doubtful, and one negative. The nuclei containing specific bodies were relatively larger than the corresponding nuclei of the human liver. Moreover, in the experimental animal the inclusions were associated with fatty and granular changes in the cytoplasm and appeared to precede or be quite independent of the Councilman lesion. In the human cases, on the other hand, when inclusions did occur, they were directly related to necrosis. In other minor respects the nuclear changes of the two species were similar.

#### OTHER CHANGES

About 80 per cent of the cases, human and animal, showed a moderate deposit of finely divided granular pigment in the parenchymal and stellate cells. It was a dull yellowish brown in color. Often the pigment lay in a fairly well defined lacuna bordering on the nucleus; at other times it was scattered freely throughout the cytoplasm. The exact nature of the pigment was obscure but it was probably of biliary origin. Attempts at differentiating hemofuscin and lipofuscin

sents itself that the demonstration of the inclusion phenomenon is dependent upon a multiplicity of factors, probably in the following order of importance: duration of illness, extent of postmortem changes, method of fixation of tissues, length of time of preservation, staining technique, and peculiarities of the virus involved.

In the control group, with one exception, we found no inclusion bodies. The exception occurred in the liver of a premature infant that had died from undetermined causes 14 days after birth. We are indebted to Dr. S. B. Wolbach of the Harvard Medical School for the tissue. The inclusions were very clear-cut and numerous, but they resembled the inclusions of herpes more than the yellow fever variety, and the associated liver damage was quite unlike that of yellow fever.

Nuclei of the parenchymal cells do not manifest pyknosis when affected by the Councilman lesion; they either pass through the stages of inclusion-body formation, or undergo an allied process whereby basophilic properties are completely lost. Such a nucleus, when incorporated within a hyaline body, shows as a dense acidophilic mass. In other conditions, where the liver cells suffer damage, pyknosis of the affected nuclei is common.

In several of our specimens we noted a nuclear change which resembled mitosis. It was confined to cells which, as far as we were able to judge, had retained their vitality even though the cytoplasm was usually granular and heavily vacuolated. The nuclear membrane was absent and the chromatin arranged in a cluster of fairly regular rod-shaped masses or granules which took a dark brownish purple stain. At times these fragments were scattered through the cytoplasm as if by an explosive process and in such instances they looked not unlike parasitic forms. At other times two irregular groups were formed so that the whole resembled a diaster phase of mitosis. Unquestionably this is the change which other investigators have interpreted as a sign of cell division, but we do not believe that it can properly be viewed as such. If there was active cell proliferation during the acute and fatal stage of the disease, as some authors would lead us to believe, we should expect to find all stages of karyokinesis, but in all our specimens we have never observed an actual transition from prophase to telophase. An occasional true mitotic figure is seen, having polar bodies and spindle threads, but the great majority of these peculiar nuclear forms show no such evidence of cell division.

fatty cells, but from this, as Seidelin has said, the conclusion is not warranted that obstruction has existed *in vivo*. In twenty-four cases, animal and human, a considerable amount of fresh blood congested the sinusoids in the midzonal regions where necrosis was most severe, but the presence of actual hemorrhage into the cord interstices was very unusual. Van Gieson's stain, employed in instances of severe parenchymal necrosis, demonstrated remarkable preservation of the fine stroma reticulum which is probably an additional factor in preventing hemorrhage from the sinusoids even after the liver cords have disintegrated. The majority of our cases presented a bloodless condition of the liver and there was a total absence of fibrin or thrombi in the blood channels.

One of the outstanding features of yellow fever is the lack of visible damage in the stroma. Herein lies one of the great differences between yellow fever and certain other liver necroses. Undoubtedly the preservation of the stroma accounts in part for the fact that there is no collapse of the lobules, no matter how intensive the damage to the epithelium may be. Occasionally the intralobular ramifications of Glisson's capsule are slightly swollen, but there is no fibrous response. In the portal areas of the African cases one sometimes encounters a moderate degree of cirrhosis which is obviously quite independent of yellow fever.

Another striking attribute of yellow fever lesions in the liver is the lack of inflammatory reaction. Scattered leucocytes are found in some liver specimens, both intra- and extravascular, more frequently in the monkey than in man, but frank infiltrations are not found in the parenchyma. Limited numbers of polynuclear and endothelial cells may appear where cellular disintegration has taken place, but unruptured necrotic cells do not appear to stimulate leucocyte migration. The point we wish to emphasize is that the liver lesion is distinctly not of the nature of a hepatitis.

The bile canaliculi in the liver can frequently be identified. They are invariably in a contracted condition and contain only minute amounts of dull, finely granular bile pigment. The bile-ducts of the portal sheaths show no damage to their epithelium beyond an occasional instance of fatty change. In a larger proportion of our human cases, slight to well marked lymphocytic infiltration was observed in the portal sheath in close relation to these biliary channels. Sometimes degenerated forms of endothelial cells were present instead of

from bile pigment were unsatisfactory and, in our opinion, of no value. Sometimes thin worm-like threads of inspissated bile could be seen within the canaliculi. The large, homogeneous globules of bright yellow bile which are often quite prominent in the livers of acute yellow atrophy cases, are not found in yellow fever.

Minute quantities of iron, located chiefly in the Kupffer cells, but present also within the liver cells and in the interstices of the portal stroma, could be demonstrated occasionally by means of Perle's stain. This pigment is to be interpreted as altered hemoglobin, and its presence is probably not related to the attack of yellow fever.

Of considerable importance is the apparent loss of glycogen from the liver as demonstrated by Best's carmine stain. Owing to the difficulty of obtaining fresh human tissues properly fixed in alcohol, it was possible to carry out the test satisfactorily on *M. rhesus* material only. Glycogen is found to disappear from the liver cells soon after the onset of the severe symptoms and when the liver is showing its early specific hyaline necrosis. Soon the liver is all but entirely depleted of its glycogen deposit. In general, the diminution of glycogen storage is proportionate to the intensity of the liver injury.

In a former communication (Klotz and Simpson) damage to the Kupffer cells of the liver in yellow fever has been dealt with at length. It will suffice, therefore, to review the important changes briefly. Hyperplasia of the stellate cells is found in the late stages of the less fulminant cases and appears to be associated with clinical jaundice. These cells are increased not only in number, but in size as well. In cases where parenchymal damage is more severe, the Kupffer cells are often swollen and granular; their nuclei may be pyknotic or otherwise degenerate. The cytoplasm frequently shows bile and iron-containing blood pigment, as well as fine hyaline droplets and, sometimes, granular fat. Occasionally these cells also show the presence of black iron-free pigment of malarial origin. The cells are commonly seen to lie loosely in the blood sinusoids, while the endothelial cells lining the sinusoids are fat and small and lie closely attached to the liver cords. In many sections the nuclei of the Kupffer cells are unusually prominent and much better preserved than those of the parenchymal elements.

In cases of severe necrosis the sinusoids may be disrupted in some part of their course, but usually they may be traced winding tortuously between jumbled cords. They are often compressed by swollen,

Special attention is directed to the non-inflammatory character of the entire liver lesion, and to the lack of autolysis in association with the necrosis. We believe that these features favor an early, complete, and scarless regeneration of the liver tissue in cases which survive the disease, and we propose to consider this aspect more fully in another article dealing with regeneration.

The identification of nuclear inclusion bodies in relation to yellow fever brings to light an unique cellular change, which so far as we know, is peculiar to virus diseases. In this respect the findings brought forward by Torres, and later by Cowdry and Kitchen, constitute an important contribution to our knowledge of the nature of the disease. It must be remembered, however, that nuclear inclusion bodies are found only in a small minority of human yellow fever cases, and that they cannot, therefore, be considered of diagnostic importance. Moreover, the positive identification of specific nuclear inclusions in yellow fever tissues is not an easy matter. Indeed, non-specific intranuclear bodies may often simulate and be confused with true inclusions so that it is not desirable in any case to have the diagnosis rest upon this distinction. Some idea of the uncertainty attending the identification of these inclusions may be gleaned from the fact that in their first publication (1929) Cowdry and Kitchen claim twenty-two out of twenty-five cases positive for inclusions, while in their later treatise (1930) they are able to list only eight of the same series as positive. If the complete microscopic picture be taken into account, there will be found ample evidence, in the human cases at least, upon which to make the diagnosis, without reference to nuclear changes.

The presence of intranuclear inclusion bodies is a diagnostic criterion of greater importance in *M. rhesus*. The Councilman lesion was present in all of our nineteen cases, but was less prominent than in the human; it is not accompanied by the same contrast in staining (acidophilic) properties, and seldom gives rise to discrete hyaline bodies. On this account it is sometimes difficult to recognize, but when found it may be taken as pathognomonic of yellow fever. It is noteworthy that cytoplasmic changes in the *M. rhesus* are less characteristic than in man, while the reverse is true of nuclear changes.

Because of failure to distinguish between Weil's disease and yellow fever, the literature has become burdened with a confusion of contradictory observations from which it is impossible to draw an ac-

lymphocytes, and occasionally both shared in the reaction. The high incidence of this lesion is striking, but its significance is obscure. We have observed the same lesion less frequently in the other forms of liver necroses. In the West African adult, irrespective of yellow fever, it is rare to find a liver that is normal in the portal zones.

## DISCUSSION

The liver lesion in the yellow fever of Africa and of America is the same and consists of a non-inflammatory degenerative process involving the parenchymal cells alone. The Councilman lesion, in our experience, is the most characteristic feature of the histological picture, and forms the best basis for a pathological diagnosis of yellow fever in the human subject. It is constantly observed and cannot be confused with other types of liver necrosis described by Mallory, Opie, McCrae and Klotz, and others.

In the early stages of necrosis the hyaline bodies of the yellow fever liver resemble the cytoplasmic inclusions of other virus diseases. In the color plate there are represented intracellular hyaline globular masses which look not unlike Negri bodies, except for the absence of basophilic granules. Not only in consistency, staining qualities and morphology does the similarity suggest itself, but the distribution in cells and the absence of inflammatory change in relation to them are characteristic of the intracellular changes in virus diseases. It is not our intention to suggest that Councilman bodies are inclusions in the same sense as those of Guarnieri, Bollinger, and Negri, but a certain analogy does exist. Later stages of the necrosis show a massive involvement of liver cells in which discrete hyaline bodies are no longer apparent and the analogy to the inclusion formation disappears.

The peculiar distribution of the Councilman lesion cannot be explained. Zonal necrosis cannot, as Chiari suggests, be accounted for by the peculiar vascular supply of the liver, for Whipple and Sperry (1909) showed that ligation of the hepatic artery and synchronous establishment of an Eck's fistula did not alter the characteristic zonal distribution of chloroform necrosis. In the yellow fever liver it is evident that we are dealing with the effect of a blood-borne toxic agent, but like the character of the agent itself, the peculiar selective localization of the necrosis is at present beyond accurate explanation.



tion played the most conspicuous role. As regards interstitial hemorrhage, there are many contradictory statements. This may have arisen from the fact that cases of Weil's disease showing hemorrhage in the liver have found their way into the yellow fever literature. Our findings agree with Sodré and Couto, Rocha Lima, Chiari, and Hudson, who emphasize the absence of hemorrhage.

The French Commission under Marchoux (1903-1906) believed that the blood capillaries in the liver parenchyma were entirely collapsed by the swelling of the liver cells; they thought this brought about an obstruction in the portal circulation which could be held responsible for gastro-intestinal hemorrhages, epigastric pain, and even the anuria of yellow fever. Their idea is merely of historical interest for there is no real collapse of the sinusoids. Rocha Lima believed that the congestion sometimes observed in regions of concentrated necrosis results from a paralysis of the capillary walls in that location.

Difference of opinion exists upon the subject of wandering cell infiltration of the liver parenchyma in yellow fever. Here, again, Weil's disease probably accounts for some of the confusion. We agree with Elliott (1920) that exudative reactions are practically absent in all uncomplicated cases.

In June, 1928, Torres drew special attention to nuclear inclusion bodies in the livers of *M. rhesus* experimentally infected with yellow fever, and was the first to suggest a specific interpretation for them, though one of us (Klotz) had previously commented upon the same nuclear bodies in a personal communication to Dr. F. F. Russell (October, 1927), comparing them to herpes inclusions as described by Goodpasture. Torres has published several well illustrated articles setting forth the character of yellow fever inclusions as found in *M. rhesus*. He has described also (1929) the finding of inclusions in one of eight human cases, a result comparable to that of Hoffmann (1929), who demonstrated the change in one of four human cases.

Cowdry and Kitchen (1929 and 1930), have established the occasional presence of nuclear inclusion bodies in the liver of the human yellow fever subject. Their more recent work, which is exhaustive and elaborately illustrated, sets forth the nature and morphology of the nuclear inclusions as seen in this disease.

Quite recently Kuczynski and Hohenadel have published some experimental work claiming to have identified a bacillus as the causa-

curate idea as to the histopathology of either disease. Many noteworthy investigators (Noguchi, Müller) have described what they thought were yellow fever lesions, when in reality the disease they were dealing with was infectious jaundice, or an allied process.

It is regrettable that the contributions of Councilman (1890) did not receive a wider circulation in the literature. To him we owe not only the first, but the best and most lucid description of the peculiar liver necrosis which is now associated with his name. He did not, however, recognize the lesion as specific for yellow fever. Since Councilman's description other authors, including Rocha Lima (1912), Turnbull (1913), Chiari (1925), Torres (1926), Hudson (1928), Couto and Rocha Lima (1929), and Hoffmann (1929) have commented upon the hyaline cytoplasmic change, but with the exception of Penna and Figueiredo (1929) no one has sufficiently stressed the differential importance of it.

The distribution of the necrosis rather than its minute character has been the more vital issue among investigators, and many excellent illustrations of zonal destruction appear in the literature. Councilman in 1890 noted that the periphery of the lobule was less affected than other parts; Carroll in 1905 made a similar observation, but Otto and Neumann in the same year asserted that islets of cells next to the central veins were the last to be destroyed. Boyce (1911) thought that necrosis was most marked in the central zone. In 1912 Rocha Lima described the midzonal necrosis as characteristic of the yellow fever liver, and this idea has been for many years the chief criterion for an anatomical diagnosis of the disease. As we have shown, it is quite fallacious to permit the diagnosis to rest upon this finding, for not only is midzonal necrosis found in other conditions, but it is frequently not to be distinguished in yellow fever. Nowadays, largely owing to the publications of Chiari (1925), Torres (1926), Couto and Rocha Lima (1929), and Hoffmann (1929), both the midzonal and the sporadic selectivity are recognized, but the essential characters of the hyaline necrosis are not sufficiently accentuated.

The earlier authors emphasized fatty changes above all other features of the yellow fever liver. Thus, Marchoux and Simond (1906) agreed with Sodré and Couto (1901) that yellow fever was virtually a "generalized steatosis." Wasdin (1898), Otto and Neumann, Carroll, Boyce, and Turnbull were all of the opinion that fatty degenera-

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tive agent of yellow fever, but the experimental lesions which they describe are not characteristic of this disease.

### SUMMARY

The gross appearance of the liver in yellow fever is characterized by the absence of distinctive changes such as are seen under the microscope.

Microscopically the liver tissue presents a non-inflammatory necrosis and necrobiosis of the parenchyma, unaccompanied by collapse of the tissue or interstitial hemorrhage. The outstanding change is a coagulative hyaline necrosis (the Councilman lesion), which does not attack the hepatic cells in an orderly fashion but occurs as a diffusely sprinkled lesion often most marked in the mid-zone (the Rocha Lima distribution). This specific necrosis is always preceded or accompanied by fatty degeneration and cloudy swelling. In the earliest stages it is characterized by the formation of dense acidophilic masses within the neutrophilic cytoplasm of the liver cells. Later, discrete, highly refractile, hyaline, globular bodies appear, often possessing a flattened pyknotic nucleus at the periphery of the mass. These bodies are usually honeycombed with fat vacuoles which they have incorporated. The process goes on to massive involvement of parenchymal tissue and ends in cellular disintegration, but no appreciable autolysis of the affected cell structures is seen in the acute stages.

The Kupffer cells suffer some damage, but not necrosis.

The vascular system, biliary channels, and stroma are uninvolved in the disease.

Specific nuclear inclusions were found in the livers of seventeen of nineteen *M. rhesus*, and twenty-three of ninety-three human cases of yellow fever. Their identification was attended with difficulties, but when their presence could be established the diagnosis was thereby facilitated.

The glycogen store of the liver is depleted in proportion to the severity of the lesion.

The liver lesions of yellow fever possess several features in common with the lesions of other virus diseases.

## DESCRIPTION OF PLATE

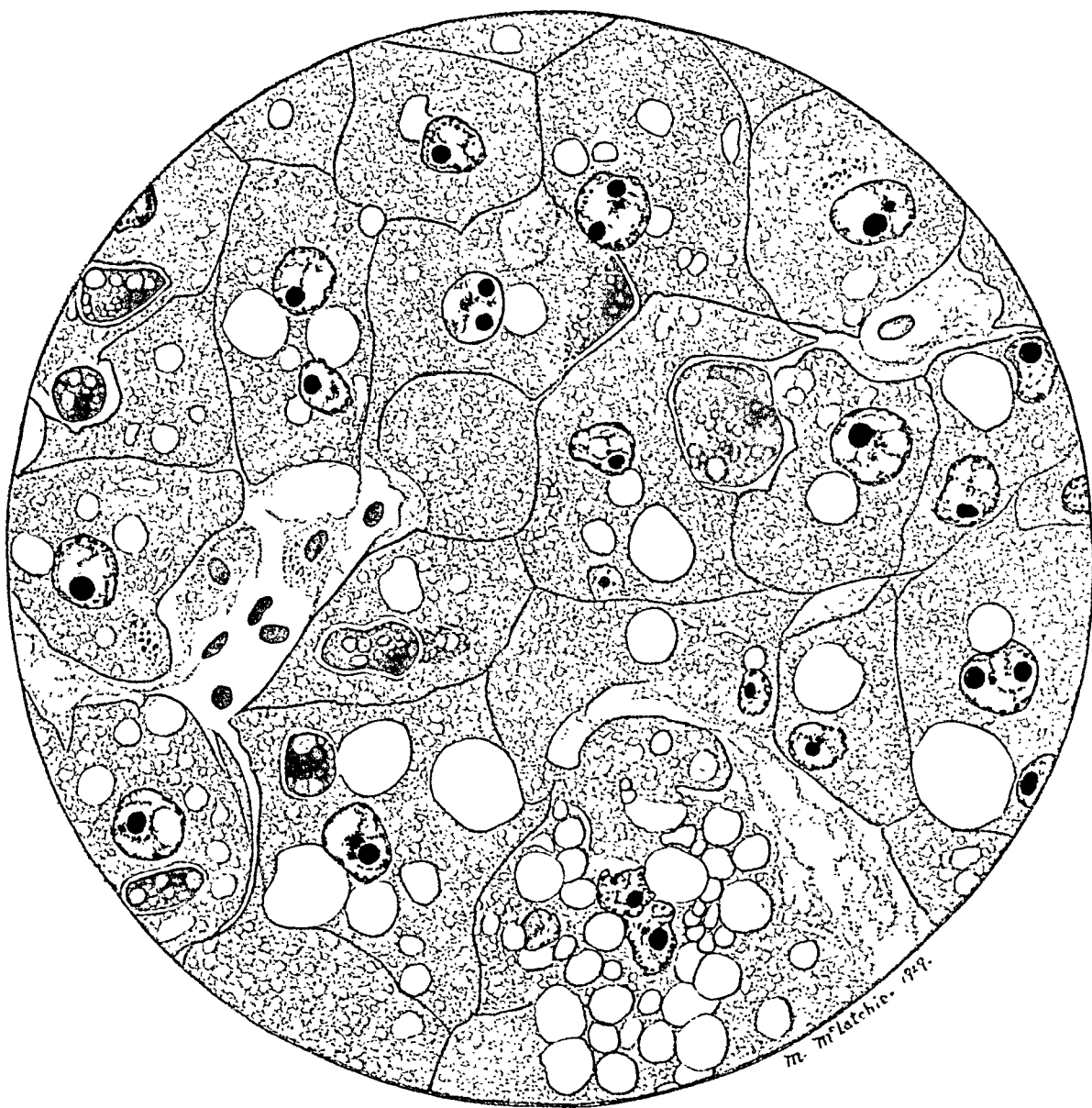
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### PLATE 126

FIG. 1. Section of human liver (magnification about 1500). Showing (*a*) Councilman hyaline lesion, (*b*) granular degeneration (cloudy swelling), (*c*) globular and granular fatty vacuolization of cytoplasm, (*d*) pigmentation, (*e*) swelling of Kupffer cells, and (*f*) non-specific nuclear degeneration.

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and the kidney in each case presented a severe tubular degeneration. The monkeys that recovered and those that died manifested similar clinical symptoms, except that in the latter the rise in temperature was of a higher and more constant character and the symptoms in general were of greater severity.

Assuming, then, that the six monkeys that recovered had sustained liver and kidney injuries comparable to those found in the fatal cases, the organs were examined with the express purpose of determining the final result of these injuries.

The autopsies revealed no unusual gross findings. Without exception, the livers and kidneys presented a healthy appearance, and showed no signs of antecedent injury. Microscopically there was an absence in all the preparations of the distinctive lesions associated with the acute phase of the disease, and scarring was not observed.

None of the livers showed increase in fibrous tissue or proliferation of biliary channels, such as is commonly seen following other types of liver injury. The lobules were of the usual size and structure, and the vascular channels were practically free from blood.

The liver cells possessed a more or less normal appearance. The nuclei were uniform, of the usual size, and well filled with chromatin, and they stained in a normal fashion. There was no indication of mitosis in any part.

In five of the six cases there was some degree of zonal differentiation. Thus, in three cases the cells of the portal zones were slightly swollen and more eosinophilic than those of other parts; the cytoplasm was finely granular and the adjacent sinusoids compressed. In the other two livers this same change was observed in the central vein areas, and the portal zones were occupied by a well marked fatty infiltration. It is possible that these zonal alterations may represent some functional readjustment to an antecedent zonal necrosis. On the other hand they may be related to an entirely irrelevant condition, such as, for instance, helminthiasis, which was present in practically all of the animals.

The liver of *M. rhesus* 6 was quite different in many respects from the livers of the other five monkeys of the series. There was no zonal differentiation. All parts of the sections showed a uniform rarefaction of the parenchymal cytoplasm and a marked edema of the cells. The sinusoids were compressed and the trabecular arrangement could not be made out. The swollen, hydropic cells were unusu-

# REGENERATION OF LIVER AND KIDNEY FOLLOWING YELLOW FEVER \*

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The present report is concerned with the study of liver and kidney tissues taken from six rhesus monkeys which had been experimentally infected with yellow fever at the laboratories of the West African Yellow Fever Commission of the Rockefeller Foundation at Yaba, Nigeria, in 1928. Each animal suffered a short but typical attack of the disease, manifesting symptoms of prostration, anorexia, fever, albuminuria and slight jaundice. Each recovered its usual health shortly after the cessation of fever, and in each case an active immunity was proved before the monkey was finally killed. The accompanying table indicates the individual histories, briefly.

*Case Histories of Rhesus Monkeys Experimentally Infected with Yellow Fever*

Monkey	Strain of virus	Incubation period	Fever	Post febrile period	Killed by
		<i>days</i>	<i>days</i>	<i>days</i>	
1.....	Asibi	2	4	16	Blow on head
2.....	A. S.	3	2	72	Ether
3.....	Asibi	5½	2	51	Blow on head
4.....	P.	5	3+	66	Blow on head
5.....	A. S.	7	2	54	Blow on head
6.....	I.	5	3	55	Blow on head

There is every reason to believe that these animals suffered appreciable damage to the liver and kidney during their attacks of yellow fever. Eighteen other monkeys, infected at approximately the same time, with the same viruses, and under the same conditions, died of the disease in periods ranging from two to eight days after the onset of fever. They showed, without exception, marked necrosis of the liver, sometimes amounting to total destruction of the parenchyma;

\* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

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there were no collections of cellular débris in the tubules. Moreover, the nuclei were healthy in appearance and indicated that the apparent cloudy change present in the cytoplasm was not associated with a lowered vitality of the cells. On the other hand, there was no definite evidence of an increased vitality or vegetative activity such as one might interpret as a sign of recent regeneration. The nuclei in cross-sections of the tubules were not increased,\* nor were there any of the epithelial giant cell formations which Oertel interprets as evidence of repair.

### DISCUSSION

It has been emphasized elsewhere (De Lamar Lectures 1927-1928), that cirrhosis of the liver does not follow yellow fever in man, and that a contracted kidney is likewise not a sequel of the disease. To this statement may now be added the weight of experimental evidence which shows that in the stage of repair, restitution of the affected organs is accomplished by complete regeneration of the functional tissues. The animals under discussion presented a practically normal histological picture of the organs except for certain irrelevant lesions. In liver and kidney alike, the restoration of the *status ante quo* was such that no sign remained to indicate the extent of regeneration or the manner in which it was accomplished.

The typical condition of the kidney in the acute stages of yellow fever corresponds in the main to a well known type of nephropathy, namely, the nephrosis of Volhard and Fahr, the "nephropathia degenerativa" of Aschoff, or more particularly the "bichloride kidney" of Elwyn. A distinction has long been made between this purely degenerative type and the inflammatory lesions of the kidney. Nephrosis is a condition met with commonly enough in acute toxemias, and it is generally recognized as a lesion which clears up completely when the toxemia passes off. The yellow fever kidney affords a very good example of a pure nephrosis, and subsequent complete regeneration is, therefore, not to be viewed as unusual or materially different from the sequence of events following nephrosis in other diseases.

The acute liver injury, on the other hand, while having several features in common with that of the kidney in yellow fever, is of a

\* According to Ribbert the average normal number of nuclei is seven.

ally clear in outline, and their closely packed arrangement lent a mosaic appearance to the sections.

Some minor changes of an indefinite character were found in the livers of all the monkeys of the series. The Kupffer cells were slightly more prominent than usual, both by virtue of their numbers, and because of the deep staining qualities of their nuclei. In several instances there appeared to be some distortion and irregular enlargement of the liver cords in the midzonal regions of the lobules. Sometimes there were four or five cells abreast in a single cord, suggesting an alteration in the original orderly arrangement of the tissue. The distortion was, however, unaccompanied by signs of karyokinetic activity or alterations in staining qualities, and it cannot be regarded as definite evidence of regeneration. The same appearance of distortion may be produced in normal liver tissue, if the microtome knife sections the lobule at an unfavorable angle. There was also in most instances slight lymphocytic infiltration of the portal sheath, a relatively insignificant lesion which occurs very commonly in monkeys that have not had yellow fever. Thus there is no obvious association between the present findings and the initial injury.

The kidneys of all the monkeys in the series presented a more or less uniform appearance, which did not deviate appreciably from the normal histological picture. The acute renal lesions associated with the fastigium of the attack and recently fully described by Magalhães, were absent. No casts were seen, no scarring nor increase in fibrous tissue and, with two exceptions, no evidence of inflammation. *M. Rhesus* 5 and *M. rhesus* 6 each presented an acute non-suppurative nephritis of a focal character, confined to the interstitial tissue. The process in both instances was of very recent origin, and apparently quite unrelated to the attack of yellow fever.

The glomeruli were not congested and the capillary tufts showed no cellular increase. The straight tubules possessed empty lumina and their epithelium was not altered.

The convoluted tubules were examined carefully, because these structures are most seriously affected in yellow fever. They contrasted in a normal fashion with the rest of the tissue, possessing the usual deep eosinophilic staining reaction. The cytoplasm was of the usual density, but finely granular. The cells were somewhat swollen and the margins of the lumina were often slightly frayed. There was no vacuolation nor desquamation of the epithelium, however, and

is probably in consequence of these facts that rapid autolysis of the destroyed cells does not occur; no dissolution of tissue is to be found during the acute and fatal stages of the disease. The injury is thus qualitatively and quantitatively limited, and as a result only the parenchymal cells suffer from the destructive process. Not only is the stroma of the portal sheaths unaffected, but the slender intra-lobular connective tissue framework, which supports the trabeculae, remains intact and can be so demonstrated by Van Gieson staining technique even when necrosis of liver cells is most intense. The absence of scarring in the yellow fever liver is to be attributed, therefore, to an absence of injury or irritation upon the stroma, which in turn must be related in part to the non-inflammatory, non-autolytic character of the degenerative process, and in part to the maintenance of a normal blood supply.

The yellow fever liver then, provides a striking illustration of the point which Mallory emphasized (1911), that destruction of the parenchymal cells alone does not, of itself, stimulate connective tissue proliferation. Whipple and Sperry (1909), and Schultz, Hall and Baker (1923) have made the same observation in connection with chloroform poisoning. Before the process of fibrosis is set in motion, the injury must involve tissue elements other than parenchymal cells, and this is true of the kidney as well as of the liver. This view is somewhat at variance with that of Kretz (1905), MacCallum (1904), and Milne (1909), all of whom consider that cirrhosis arises in consequence of a primary destruction of liver cells.

We have indicated the conditions associated with the absence of fibrosis in the yellow fever liver. For a discussion of the factors which incite fibrosis in other diseases, the reader is referred to Mallory (1911), Pearce (1904, 1906), Muir (1908), Milne (1909), Opie (1910), Rolleston (1912), Herxheimer and Gerlach (1921), Schultz, Hall and Baker (1923), Hall and Ophüls (1925), Roman (1927), MacMahon and Mallory (1929), and MacMahon, Lawrence and Maddock (1929).

Our investigations of a large series of fatal cases of yellow fever in man and monkey show that active regeneration of liver and kidney tissues is practically at a standstill during the toxic phase of the disease. The series of animals that recovered, which we report here, shows however, that regeneration is complete within sixteen to seventy-two days after the cessation of fever. Therefore, the rep-

distinctive character, as we have been at some pains to show in a previous communication; and in consideration of the severe and extensive necrosis it is noteworthy that complete and scarless restitution of the liver takes place in cases of recovery. Clinical experience with yellow fever shows that it differs from similar pathological processes of equal intensity in the matter of freedom from sequelae in the liver. Various kinds of hepatorenal poisonings, as for instance, arsenic, phosphorus and carbon tetrachloride poisonings, and some toxemias of pregnancy, have a tendency to produce permanent liver damage, which not infrequently progresses into the fatal stages of acute yellow atrophy (Roman, 1927). Similarly, infective processes such as catarrhal jaundice and some obscure forms of hepatitis lead to marked scarring of the liver. In yellow fever, on the contrary, both clinical and experimental evidences tend to show that normal hepatic function is quickly restored in individuals that recover, and that these individuals never suffer cirrhosis of the liver or acute yellow atrophy as a consequence of their attack.

What are the factors which favor this peculiar power of regeneration? Undoubtedly the self-limiting character of the infection is of importance. Not only is the toxemia of relatively short duration, but there is no recurrence after the initial attack, no repetition of injury such as is commonly held to account for the sclerosing diseases. Yet in yellow fever the single injury is probably often of greater magnitude than the sum total of repeated injuries which in other diseases produce sclerosis. Thus the fact of a short period of injury is not a sufficient explanation. We must look to some peculiar features of the pathological process in yellow fever for an interpretation of the end results.

Inquiry into the absence of fibrous response in the yellow fever liver leads to a consideration of the character of the initial lesion. As we have pointed out elsewhere, yellow fever *per se* does not induce an inflammatory response. There is no exudative response to the injury and no appreciable migration of leucocytes to the damaged tissues. The Councilman necrosis of the liver, peculiar to this disease, is coagulative rather than autolytic, and since leucocytes are not attracted to the foci of necrosis, there is, presumably, no pouring out of proteolytic substances. The vascular channels, both extra- and intralobular, remain free from thrombi so that the circulation is not interrupted, and no digestion of tissue arises through infarction. It

in partial extirpation experiments, as might be expected, are not to be found in the yellow fever kidney.

### SUMMARY

Six rhesus monkeys which had recovered from experimental yellow fever showed complete and scarless regeneration of the liver and kidney. This bears out clinical evidence that neither cirrhosis of the liver, nor contracted kidney follows yellow fever in man.

Special attention is directed to the sequence of events taking place in the liver. Except in cases of chloroform poisoning, liver damage of equal magnitude rarely occurs without producing some scar formation. The yellow fever liver proves that destruction of parenchymal cells alone is not a sufficient stimulus to induce replacement fibrosis.

The absence of fibrosis in the liver and kidney is due to a peculiar immunity which the stroma structures manifest toward the yellow fever injury; there is no stimulation of connective tissue elements during the acute stage of the disease. The reasons for this are, we believe, related to the non-inflammatory, non-autolytic character of the acute pathological process, and to the absence of thrombosis in the small parenchymal vessels.

Regeneration originates in islands of parenchymal cells which have survived the attack, and quickly restores the tissues to their original state.

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arative process must go on to completion within the ordinary period of convalescence.

In the case of the liver, it is not difficult to imagine how breaches of continuity in the parenchymal cords are bridged over by proliferation of liver cells which have survived the toxemia. The slender cylindrical stroma encasements of the trabeculae are preserved and the growing cells spread by direct extension inside of these limiting membranes to replace the cells which have been destroyed and absorbed. Thus, the original pattern of the tissue is restored. All investigators of repair in the liver agree that the chief rôle in the regeneration of the parenchyma is played by old, undamaged liver cells. MacCallum (1902) observed that "where well-differentiated liver cells still persist the new liver tissue is very simply produced by their mere multiplication by division, and the less highly differentiated gall-ducts take no part in the process, but remain quiescent in their subordinate position as conductors of the secretion of the liver cells." The much debated question as to whether liver cords may take their origin from biliary epithelium does not arise in connection with the present study, for, as we have seen, there is no proliferation of the bile ducts in the tissues under discussion. It is only when the supporting stroma has been damaged and stimulated to proliferation, as in the case of acute yellow atrophy and in the cirrheses, that attempts at regeneration take the peculiar distorted form of pseudobile ducts or pseudotubules and nodular hyperplasia. This type of regeneration, fully reviewed by Hess (1913), Blum (1923), Roman (1927), and Fishback (1929), does not concern us in connection with yellow fever.

In the kidney, regeneration of the convoluted epithelium is no doubt accomplished largely by proliferation of cells which survive the attack. Islands of living cells always remain from which a new lining may originate. In certain cases of extensive necrosis, it is probable also that the cells of the straight tubules may grow in to replace the destroyed secretory epithelium, assuming its morphology and its specialized function. As in the case of the liver, the intact basement membranes which remain unaltered in the disease orientate the new cells into the old pattern, as proliferation and extension take place. The functionless regeneration phenomena described by Oertel (1909) in chronic nephritis, and by Podwyszożki (1887), Ribbert and Peipers (1895), Thorel (1907), and Pearce (1909)





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acetic acid. The resulting precipitate was then washed and inoculated. Such inoculations always produced typical lesions, while portions of the tissue treated with potassium hydroxide, washed, and inoculated without acetic acid treatment, gave uniformly negative results.

Since the method of extracting tissue with potassium hydroxide and then precipitating with acetic acid is one employed in preparing nucleoproteids, Sanfelice assumed that he had isolated a nucleoproteid agent responsible for the disease. This agent, according to his hypothesis, is a toxic material which has the properties of an acid, being inactive or neutralized when combined with potassium hydroxide, but readily liberated upon the addition of an excess of acid. Such a non-living, toxic nucleoproteid, he proposed, is elaborated by the affected cells, and, upon transmission to other cells, causes these in turn to elaborate more of the same agent.

Sanfelice's argument that no living agent could be responsible for "takes" at twenty-four hours is based upon earlier work, which, he says, was corroborated by Burnet.\* In 1897, at a time when he held that the agent of pigeon-pox was a blastomyces,<sup>9</sup> Sanfelice tested the toxic effects of several chemicals and disinfectants upon the virus. Among these were potassium hydroxide, acetic acid, and phenol. He found that the virus was killed after five minutes in 0.5 and 1 per cent potassium hydroxide, 1 per cent acetic acid, and 0.5 and 1 per cent phenol. In these experiments, however, Sanfelice employed a very difficult technique. Silk threads, immersed in pigeon-pox material, were dried at 37° C, then immersed in 1 per cent potassium hydroxide for five minutes, washed in sterile water, and finally scraped to obtain material for inoculation. This procedure would, we believe, be difficult to perform without many chances for the complete loss of the virus; and the results should not be used as a basis for comparison with other experiments in which larger amounts of infected tissue were employed.

Sanfelice has by no means gained universal acceptance of his hypothesis. Friedberger<sup>10</sup> in 1918 found no evidence that virus "inactivated" by potassium hydroxide could be reactivated by acetic acid. He reported that pigeon-pox virus in dilute suspension was

\* Burnet<sup>7</sup> merely refers to a paper by Reischauer<sup>8</sup> in which Sanfelice's original work is quoted.

# THE NATURE OF FOWL-POX VIRUS AS INDICATED BY ITS REACTION TO TREATMENT WITH POTASSIUM HYDROXIDE AND OTHER CHEMICALS\*

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Recent experiments,<sup>1,2</sup> have given additional morphological evidence that the inclusion bodies found in the infected epithelial cells of a fowl-pox lesion represent colonies of a minute microorganism (Borrel bodies<sup>3</sup>) embedded in a ground substance of lipoproteid composition. Following this work, the highly infectious nature of isolated inclusion bodies was demonstrated by Woodruff and Goodpasture.<sup>4</sup> In further experiments,<sup>5</sup> it has been shown that a fraction of an inclusion will produce a typical fowl-pox lesion upon inoculation. This demonstration of the high degree of infectiousness of the inclusion bodies, and the definite morphology of their Borrel body components aroused our interest in the work of previous investigators which led them to believe that the virus of fowl-pox or pigeon-pox is a non-living agent. In particular, the experiments of Sanfelice attracted our attention since they are so frequently quoted in the literature as indicating the non-viability of at least one of the viruses. For this reason Sanfelice's work was repeated with care and will be described in some detail.

Sanfelice used pigeon-pox for his experiments. The virus of pigeon-pox is infectious for the chicken and induces a lesion identical with that of the strain of virus indigenous to fowls. Sanfelice,<sup>6</sup> by treating the virus of pigeon-pox with 1 per cent potassium hydroxide, claims to have extracted a nucleoproteid toxin which would produce typical lesions. His technique was to grind thoroughly a piece of the fresh tissue from a pigeon-pox lesion and to add 1 per cent potassium hydroxide amounting to two or three times the weight of the tissue. After standing four to twenty-four hours and longer with the potassium hydroxide, the material was filtered through a piece of linen and the filtrate treated with twice its volume of 1 per cent

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washing, as well as by precipitation with acid, produced typical lesions upon inoculation.

Following these experiments in which the effect of potassium hydroxide was determined on comparatively large particles of tissue, an attempt was made to obtain suspensions which might contain only that most minute element of the fowl-pox lesion, the Borrel body. A series of experiments was started using the dried scabs from fowl-pox lesions. These were carefully ground in distilled water and then the larger particles were thrown down by centrifug-

TABLE I

*Effect of 1 per cent Potassium Hydroxide on Finely Divided Suspensions of Virus*

Virus Suspensions		Time at which Virus was Tested				
Infectious at dilution of	Dilution employed in KOH experiments	2 min.	$\frac{1}{2}$ to 4 hrs.	9 hrs.	16 hrs.	19 to 24 hrs.
1:1000	1:2	..	..	..	..	—
1:1000	1:2	..	..	..	..	+
1:1000	1:2	+++	..	..	..	—
1:1000	1:2	+++	..	..	..	—
1:1000	1:2	..	..	..	..	—
1:100	1:2	+	..	..	..	—
1:2000	1:2	+++	+++	..	..	..
1:2000	1:2	..	+++	..	..	..
1:2000 *	1:2	+++	+++	..	..	..
1:2000 *	1:2	..	+++	..	..	..
1:2000 **	1:2	..	+++	..	..	—
1:2000 **	1:2	+++	+++	..	..	..
1:2000 **	1:2	+++	..	+++	++	+

\* = filtered  
 \*\* = filtered (Whatman 42)  
 +++ = massive lesion

++ = several nodules  
 + = one or two nodules  
 — = negative

ing fifteen to twenty minutes at low speed. The fine supernatant suspension was decanted, and in some instances filtered through filter paper. In one or two experiments, especially fine filter paper (Whatman 42) was used. While we cannot say definitely that the resulting filtrate was a Borrel body suspension, yet we are certain that the precaution taken excluded any large inclusions, and that, in addition to Borrel bodies, only very small or fractional inclusions were present. In order to test the virulence of this filtrate, part of it was used for inoculation at various dilutions. The rest was mixed with equal quantities of 2 per cent potassium hydroxide to give a

killed, as was *B. prodigiosus*, after five minutes treatment with 1 per cent potassium hydroxide. He then employed Sanfelice's technique and obtained typical lesions after treatment of virus fourteen hours in 1 per cent potassium hydroxide, not only with inoculations of the "nucleoproteid" precipitate, but with control inoculations of filtrate treated with potassium hydroxide alone. Friedberger stated that Sanfelice's linen filter let through large particles of tissue which no doubt resisted the action of 1 per cent potassium hydroxide longer than the dilute suspension that he himself used in comparing its viability with that of *B. prodigiosus*. Sanfelice still upheld his nucleoproteid hypothesis, however, and in 1927 produced another paper<sup>11</sup> in which the refutation of Friedberger was attempted.

In our own experiments to test the effect of 1 per cent potassium hydroxide on the virus, dried rather than fresh scabs from the lesion of fowl-pox were used, and a filter of absorbent cotton rather than linen. Otherwise Sanfelice's technique was followed exactly. The dried scabs were ground in a mortar with distilled water or saline, and the resulting suspensions were found to contain virus in such concentration that, when inoculated on a chick, positive results were obtained in dilutions as great as 1:1000. The suspensions were then mixed with equal quantities of 2 per cent potassium hydroxide, and these suspensions of fowl-pox material in 1 per cent potassium hydroxide were allowed to stand at room temperature for periods of four and ten hours. After such treatment, part of the material was filtered through absorbent cotton, and precipitated with twice its volume of 1 per cent acetic acid. The precipitate was then washed and inoculated. Inoculations after four hours produced only small lesions while those after ten hours were negative. At the same time, some of the sediment from the potassium hydroxide suspension, thrown down by centrifuging and then carefully washed, was found upon inoculation to produce massive lesions after both four and ten hours. These experiments seemed to point directly to the size of the particles as being an important factor in the protection of virus from the action of the potassium hydroxide, since in the centrifuged sediment the particles were obviously larger than those in the filtrate. We noted also in an experiment with unfiltered material, that inclusion bodies were present in the tissue. After treatment of this material with 1 per cent potassium hydroxide for twelve and twenty-four hours, sediment freed from the potassium hydroxide by merely

placed in sterile test tubes and centrifuged. The inclusion sediment was washed with sterile saline, and inoculated either into feather follicles or on defeathered, scarified areas.

Table II shows that inclusions will resist the action of 1 per cent potassium hydroxide for from one to five days, the size of the lesion produced diminishing with length of treatment. The massive lesion produced by bodies treated twenty-four hours is shown in Fig. 3. Fig. 5 shows the smaller lesions produced by bodies treated seventy-two hours. It was found that different specimens varied in their ability to resist the action of 1 per cent potassium hydroxide. In-

TABLE II

*Virucidal Effect of 1 per cent Potassium Hydroxide on Inclusion Bodies*

Time in KOH	16 to 24 hrs.	2 days	3 days	4 days	5 days	6 days
No. of inoculations . . . . .	23	11	13	8	5	2
No. of positives . . . . .	23	8	8	1	2	0
Type of Lesion. . . . .	23	..	..	..	..	..
{ +++	..	6	1	1	..	..
{ ++	..	2	7	..	2	..
{ +	..					

+++ = massive lesions.

++ = several nodules.

+ = one or two nodules.

clusions obtained from a fowl-pox lesion more than ten days old seemed more fragile and more readily damaged than those from a seven- to ten-day lesion. In these experiments it will be noted that mere washing after potassium hydroxide treatment was sufficient to prepare bodies for inoculation. While neutralization of the potassium hydroxide with acetic acid was effective, the simple washing of the inclusions with saline gave just as large lesions after just as long periods of treatment as did the acetic acid method. After treatment for a sufficient length of time, varying from two to six days, neither washing nor neutralizing with acetic acid was effective in "reactivating" the virus.

Since recent work points to the Borrel body as the probable etiological agent of fowl-pox, it seemed of interest to determine whether Borrel bodies could still be identified in inclusions which had been proved infectious after a long period in potassium hydroxide. It was found that after treatment with potassium hydroxide, the physical characteristics of the inclusion bodies were altered. A jelly-like substance developed about each body causing groups of bodies

1 per cent concentration, and allowed to stand at room temperature. With this finely divided material, the only means of freeing the virus from potassium hydroxide was to neutralize with acetic acid and wash. This we did, finding that the protein precipitate which followed addition of acid carried down with it any active virus, while the supernatant fluid was generally innocuous.

Table I shows that virus inoculated after two minutes treatment produced massive lesions. Similar lesions were obtained up to four hours, and in one instance at nine hours (see Fig. 1). The length of resistance probably varied with the fineness of the suspension. Two lesions of one or two nodules were obtained in the nineteen to twenty-four hour period (see Fig. 2), but the majority of these inoculations were negative.

The important point in the above experiments is the fact that the suspension of virus showed evidence of being progressively destroyed — the longer the treatment, the smaller the lesion. There was no suggestion whatever that virus once inactivated could be "reactivated" by means of acetic acid. A few experiments, in which fresh tissue from the fowl-pox lesion was used instead of the dried tissue, indicated that resistance might be somewhat longer with fresh material than with the dried. However, the fresh material too showed evidence of being progressively diminished in virulence rather than of having any ability to be "reactivated" at full strength after long periods of treatment.

Since the above experiments indicated that the size of the particles which were treated with potassium hydroxide was an important factor in determining the resistance of the virus, it seemed logical to make use of the fowl-pox inclusion bodies in further work. As has been demonstrated,<sup>4</sup> these inclusions are highly infectious and can be obtained free from cellular material by tryptic digestion. Accordingly, suspensions of inclusion bodies were obtained by tryptic digestion. The inclusions were removed by centrifuging and were washed in saline or distilled water. Equal volumes of inclusion body suspensions and 2 per cent potassium hydroxide were mixed in order to obtain suspensions of inclusions in 1 per cent potassium hydroxide. These were treated for periods of from one to six days, the material being transferred to fresh sterile test tubes every twenty-four hours to insure uniform action of the chemical. At the end of these treatments, the suspensions were carefully removed,



filtration of ground material in 1 per cent potassium hydroxide is slower, due to the viscidness of the suspension, still the possibility of small inclusion bodies passing through the linen filter is obvious, and, as we have shown, these may be still viable.

In a further series of experiments, an attempt was made to extract a toxic nucleoproteid from inclusion bodies, such as that described by Sanfelice. Since inclusions are so highly infectious, a specific toxin in the sense of Sanfelice might be expected to be present in

TABLE III

*Toxin Theory Tested with Inclusion Bodies*

Filtrate of supernatant fluid infectious at dilution of		Filtrate of supernatant fluid x6 to 24 hours in KOH	Sediment of inclusion bodies x6 to 24 hours in KOH
A	1:5	—	+++
	1:2	—	+++
	1:2	—	+++
	1:2	—	+++
	1:2	—	+++
	1:2	—	+++
B		—	+++
		—	+++
		—	+++

+++ = massive legion.  
— = negative.

quantity within them. Saline suspensions of the digested bodies were made as previously described. These were allowed to stand about half an hour in order to obtain in the saline some active virus, free from inclusions. The suspension was then centrifuged and the top portion of the supernatant fluid filtered. This filtrate contained very weak virus, active in dilutions not greater than 1:2 to 1:5. The weak filtrate and the inclusion body suspension were treated separately with 1 per cent potassium hydroxide for long periods, sixteen to twenty-four hours. It should be stated that the potassium hydroxide and acetic acid were added to proportionate parts of the

to stick together. Within this film, the definite outline of the compact inclusion was apparent. When the usual technique<sup>5</sup> of drying out of distilled water was applied to these inclusions, they failed to break up and liberate their Borrel bodies. Moreover, in stained preparations, such inclusions appeared to have hard, incrustated surfaces, and it seemed that some further means was necessary to break up the inclusions after potassium hydroxide treatment. After some experimenting, it was found that rupture of the inclusions could be brought about mechanically by means of a small glass point. Fig. 8 shows a smear of Borrel bodies obtained from inclusions which had been in 1 per cent potassium hydroxide for twenty-four hours, then washed in distilled water and scratched with a fine glass point. Fig. 9 shows an inclusion stained after such treatment. One side of the inclusion has been ruptured, and the contents have poured out in fan-shape. Characteristic Borrel bodies are shown at the edge of the mass where the bodies are more widely dispersed. In Fig. 10, the same inclusion is shown with the broken edge of the surface of the inclusion in focus. The granular structure of the extruded contents and of the material remaining within the inclusion may be seen. The broken edge of this inclusion gives one the impression that the outer surface is hardened and shell-like. Such an incrustation of the inclusions may be responsible for their long survival in potassium hydroxide, though no data have been obtained as to which component of the lipoproteid ground substance of the inclusion reacts with the potassium hydroxide to produce this hardening.

A possible explanation for the "reactivation" obtained by Sanfelice after twenty-four hours in potassium hydroxide is that the linen filter which was used by him allowed larger particles of the diseased tissue to pass than did our absorbent cotton filter, or fine filter paper. These larger particles were, we judge, responsible for the successful inoculations rather than any nucleoprotein extract. This possibility has been suggested also by Friedberger, but he did no further work to determine the actual viability and structure of such large particles in potassium hydroxide. In the grinding of fresh material it was found impossible to break up completely all the masses of inclusion bodies present in the diseased tissue. Many inclusions are freed by grinding but are not further injured by ordinary grinding methods. Furthermore, digested inclusions pass readily through a linen filter such as Sanfelice described. While the

were positive and, following the second procedure, negative. According to his own description, however, his technique was varied slightly in the two cases. In the first case the ground material was mixed with saline, filtered through ordinary filter paper and the filtrate mixed with 2 per cent potassium hydroxide. In the second case the ground material was mixed directly with 1 per cent acetic acid and this mixture filtered. The results which we have obtained in repeating this experiment indicate that the direct addition of acetic acid to the ground material forms a sticky mass which does not filter as readily as the saline suspension. It would seem, therefore, that Sanfelice's reported results are due to the passing of copious virus when the saline suspension was filtered, while the major portion of the virus was withheld from the filtrate when the sticky acid suspension was filtered.

In the course of this work with potassium hydroxide and virus, it was noted that inoculations made without first removing the potassium hydroxide showed severe scabbing. Tests were made to determine the action of 1 per cent potassium hydroxide alone on chicken epithelium. Epithelial tissue, treated with a single application of 1 per cent potassium hydroxide and excised after twenty-four hours, shows, in stained sections, that the epithelial cells have been completely destroyed (see Fig. 7). Experimental inoculations with active virus in potassium hydroxide have in several instances produced no lesions, and, in other instances, it has been noted that smaller lesions are produced than with an equivalent amount of virus from which the potassium hydroxide has been removed (see Figs. 4 and 3). Thus, destruction of epithelial cells may be the cause of negative results in experiments where the potassium hydroxide is not removed, since epithelial cells are necessary for the proliferation of the virus. Possibly the existing disagreement as to potassium hydroxide effects may be due in part to insufficient control of this factor.

A few further experiments on the virucidal action of chemicals have been carried on. In two experiments inclusion bodies were found still viable after two hours in 1 per cent phenol, in contradistinction to Sanfelice's report that the virus is killed after five minutes in 0.5 per cent and 1 per cent phenol.\* The discrepancy here

\* In a recent paper Kligler<sup>12</sup> reports that fowl-pox virus remains viable after fifty days in 0.25 per cent phenol.

weak virus suspension in such a percentage that the total dilution of this fluid was no greater than 1:2. After neutralization, the filtrate was inoculated. Results with the filtrate after potassium hydroxide and acetic acid treatment were uniformly negative, while inclusion bodies which were merely washed free of potassium hydroxide but were not treated with acetic acid always produced massive lesions (see Table III-A and Fig. 6).

The above experiment seemed fairly conclusive that the small amount of active virus present in a suspension free from inclusions is not a nucleoprotein toxin, for otherwise it could, according to Sanfelice's hypothesis, be reactivated by acetic acid treatment. In this experiment, however, we did not allow for the possibility that potassium hydroxide might be more effective than saline in extracting such a toxin from the inclusions. Therefore, we again prepared suspensions of digested and washed inclusions, placed them in 1 per cent potassium hydroxide, and after one-half hour, centrifuged to throw the inclusions to the bottom in order that they should not become mixed with the supernatant fluid and confuse the results. The whole amount of material was then allowed to stand with the potassium hydroxide for twenty-four hours. In this way any hypothetical nucleoprotein toxin present in the inclusions could be dissolved into the supernatant fluid. After twenty-four hours, portions of the supernatant fluid were filtered, neutralized with acetic acid, and inoculated. Table III-B shows that in three experiments this filtrate was inactive, while the sediment of inclusion bodies, washed only, produced massive lesions. This we judged, was sufficient evidence that no toxic product could be extracted, by Sanfelice's method, from inclusions, highly infectious as they are. In all respects, inclusions respond to potassium hydroxide treatment by a progressively diminishing strength, being capable of resisting the action of 1 per cent potassium hydroxide for several days, but once inactivated, no treatment with acetic acid is capable of "reactivating" them. We have been able to obtain no evidence in support of the nucleoprotein toxin theory.

In a paper<sup>11</sup> which appeared in 1927 refuting Friedberger's work, Sanfelice cited experiments in which carefully ground, diseased tissue was treated in one case with potassium hydroxide followed by acetic acid, and in the second case with acetic acid followed by potassium hydroxide. His inoculations made following the first procedure

nite period of time. This period varies, depending upon the physical state of the virus. Suspensions of very finely divided material, we found, were progressively destroyed, being diminished in strength after several hours and completely inactive after twenty-four hours. Inclusion bodies, on the other hand, will resist the action of 1 per cent potassium hydroxide for from one to five days. This greater resistance of the inclusions is due, we judge, both to the great concentration of virus within them and to the protection offered by their lipoproteid supporting substance. Moreover, stains of inclusions which were proved to be still viable after twenty-four hours treatment with potassium hydroxide show that Borrel bodies are still present.

In attempts to extract a nucleoproteid toxin from inclusion bodies, freed from cellular material by digestion, we have found no evidence of the existence of a transmissible toxin. There is very little free virus in the fluid portion of a saline suspension of digested inclusions, and none if each inclusion is washed separately.<sup>4</sup> The small amount present in our suspensions never survived twenty-four hours treatment with 1 per cent potassium hydroxide, whereas inclusion bodies thus treated did not lose their infectiousness for a much longer period. This result indicates that the active virus of an inclusion is an integral part of the inclusion and not superficially adsorbed by it. If the virus were held merely on the surface of inclusion bodies, inclusions might be expected to lose their activity in potassium hydroxide as rapidly as free virus.

The fact that inclusions remain viable after long periods in potassium hydroxide, this viability being presumably dependent upon the survival of materials within the inclusion, together with the demonstration of the presence of Borrel bodies in inclusions after twenty-four hours treatment with potassium hydroxide, supports the evidence of Goodpasture,<sup>1, 2</sup> and Woodruff and Goodpasture,<sup>4, 5</sup> that the inclusions of fowl-pox are colonies of a minute microorganism, the Borrel body.

#### SUMMARY

1. Fowl-pox virus in suspensions of finely divided material is rendered inactive after a period of four to twenty-four hours in 1 per cent potassium hydroxide. In the form of inclusion bodies, however, the virus is found to be infectious, in diminishing strength, after treatment with potassium hydroxide for as long as five days.

may well be due to the different form of the virus — in one case inclusion bodies and in the other case finely ground material dried on silk threads. Our results indicate that the virus, when protected in the intact inclusion body, is very resistant to the chemical agents we have used.

Extraction of dried virus with alcohol, followed by ether, and with ether alone, for periods of one-half to one hour does not destroy the activity of the virus. Virus treated thus has proved active in dilutions as great as 1:1000. Inclusions dried and treated with ether for one-half hour kept their form and activity, though fat stains showed that most of the lipid had been removed.

### DISCUSSION

Publications of Sanfelice,<sup>6, 11</sup> describe a technique for the isolation of a toxic nucleoproteid from the infected epithelium of a pigeon-pox lesion. Such a toxin he claims is capable of reproducing the typical lesion. After treatment of virus with 1 per cent potassium hydroxide, Sanfelice's technique requires the addition of 1 per cent acetic acid to "reactivate" the virus. Since this procedure is the usual one for the isolation of nucleoproteids, Sanfelice considered the activity of virus following treatment with potassium hydroxide to be due to the extraction of a toxic nucleoproteid. We have found that removal of the alkali by merely washing the tissue which has been treated with potassium hydroxide, is just as effective in obtaining active virus as is the method of precipitating with acetic acid. This work corroborates Friedberger's experiments.<sup>10</sup> The active material obtained after treatment with potassium hydroxide is therefore not necessarily a nucleoproteid derivative. Furthermore, after a sufficient length of treatment with potassium hydroxide, the virus becomes completely inactive, and cannot be "reactivated" by any method.

Thus our experiments lead us to make an interpretation, differing from that of Sanfelice, concerning the nature of the virus. Sanfelice considered that the virus had the properties of an acid, being inactive or neutralized when combined with potassium hydroxide, but readily liberated upon the addition of an excess of acid, while we have found that the virus is not inactivated immediately upon the addition of 1 per cent potassium hydroxide, but survives for a defi-

## DESCRIPTION OF PLATES

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### PLATE 127

- FIG. 1. Massive fowl-pox lesion produced by inoculation of precipitate from Borrel body suspension after nine hours in 1 per cent potassium hydroxide.
- FIG. 2. Two small nodules on left edge of scar show the diminished infectiousness of the precipitate from a Borrel body suspension after nineteen hours in 1 per cent potassium hydroxide.
- FIG. 3. Massive fowl-pox lesion produced by inoculation of inclusion bodies after twenty-four hours in 1 per cent potassium hydroxide.
- FIG. 4. Result of the inoculation of same preparation of inclusions as used in Fig. 3, but without removal of the alkali.
- FIG. 5. Small lesion produced by the inoculation of inclusion bodies after seventy-two hours in 1 per cent potassium hydroxide.
- FIG. 6. The massive lesion on the right was produced by the inoculation of inclusion bodies after twenty-four hours in 1 per cent potassium hydroxide. The non-infected scar within the square on the left shows the negative result obtained by inoculation of free virus suspension after treatment for the same period.

2. No evidence has been found for the existence of a nucleoprotein toxin, such as that described by Sanfelice, either in scabs of the fowl-pox lesion or in digested inclusion bodies.

3. The destructive action of 1 per cent potassium hydroxide on normal epithelial cells of the chick is shown.

4. The presence of Borrel bodies in inclusions which have been proved infectious after remaining twenty-four hours in 1 per cent potassium hydroxide has been demonstrated.

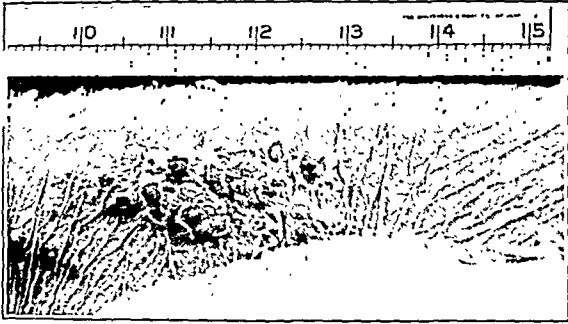
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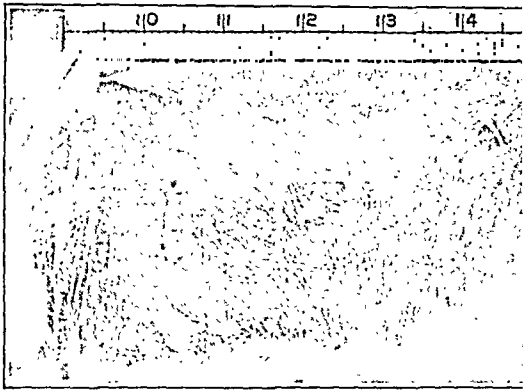


PLATE 128

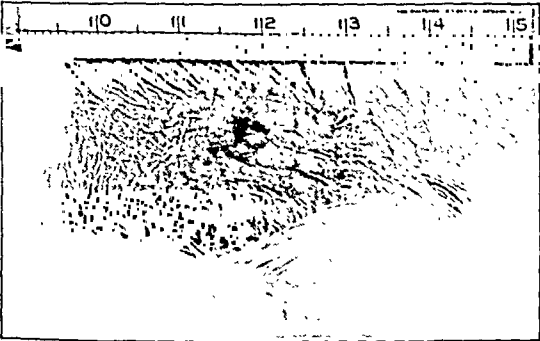
- FIG. 7. Complete destruction of epithelial cells of skin of chick after short application of 1 per cent potassium hydroxide.
- FIG. 8. Borrel bodies from inclusions which had remained twenty-four hours in 1 per cent potassium hydroxide. Morosow's stain. Photomicrograph taken with blue light.  $\times 1860$ .
- FIG. 9. An inclusion body which had remained twenty-four hours in 1 per cent potassium hydroxide, mechanically ruptured to free the Borrel bodies. Morosow's stain. Photomicrograph taken with blue light.  $\times 1860$ .
- FIG. 10. Same ruptured inclusion as shown in Fig. 9 with focus on outside surface of body. Morosow's stain. Photomicrograph taken with white light.  $\times 1860$ .



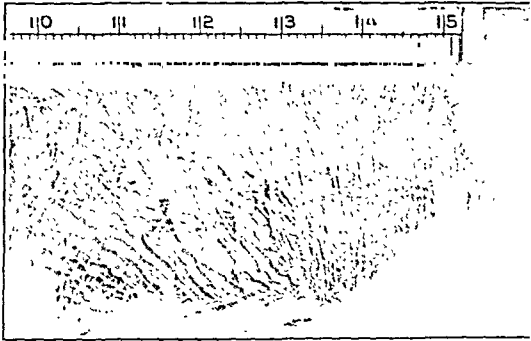
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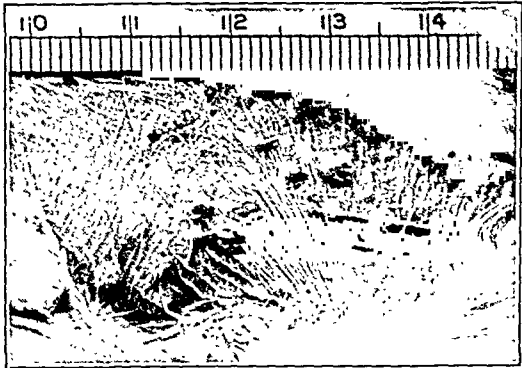
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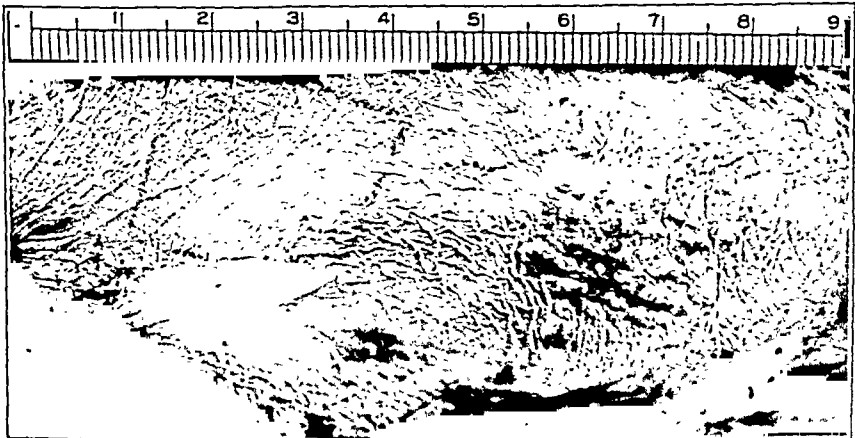
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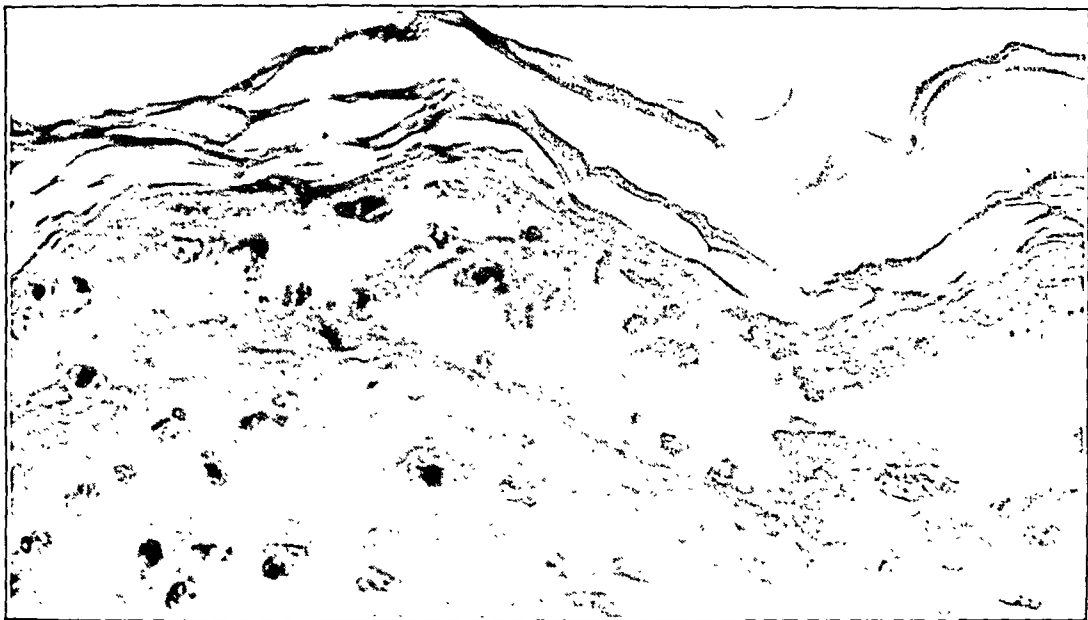


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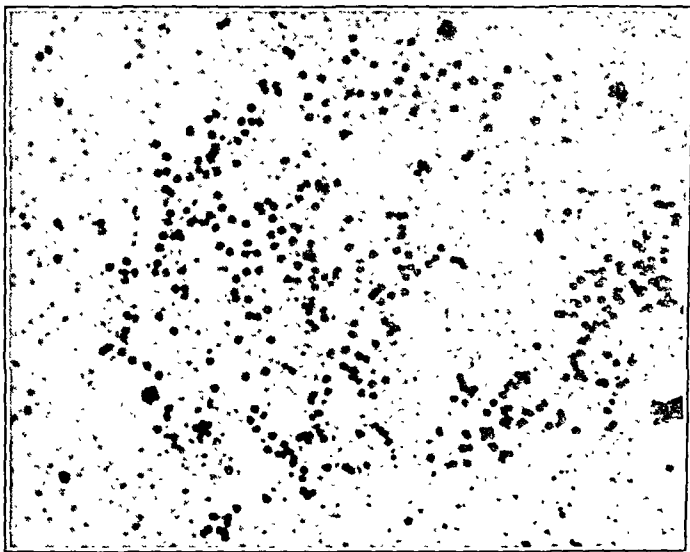


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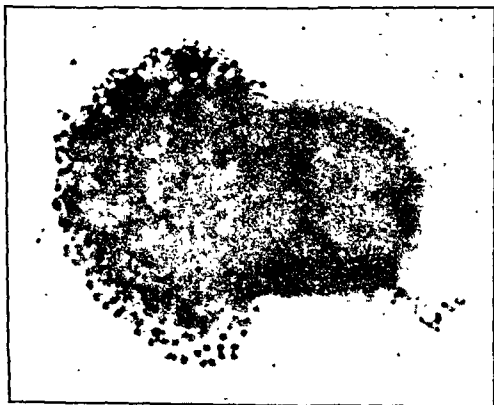




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structures 0.25 of a micron in diameter. These structures have been called Borrel bodies, after their discoverer. Borrel suggested that the inclusions of fowl-pox might be made up of these minute coccoid structures, and in 1906 Burnet<sup>6</sup> demonstrated in sections of the fowl-pox lesion stained in one case by Giemsa's method and in the other case by Loeffler's flagella stain, that the inclusions and the "micrococcal masses" belong to the same cells. In 1928 it was shown by Goodpasture<sup>7</sup> that the inclusion bodies of fowl-pox, when suspended in saline, appear as compact, hyaline structures, but that upon being washed and suspended in distilled water the same bodies swell markedly and develop vacuoles in which may be seen minute granules dancing about in rapid Brownian motion. These granules, as demonstrated by crushing an inclusion body on a slide, were shown to be Borrel bodies.

The marked change which occurs in fowl-pox inclusion bodies upon immersion in distilled water is shown in Figs. 1 and 2. In Fig. 1 may be seen eight inclusions in saline while Fig. 2 shows the same eight inclusions at the same magnification thirty minutes after distilled water had been substituted for the saline. There has been an increase in the linear dimensions of the bodies of one-third to one-half, indicating a volume increase of two to three times. One of the swollen inclusion bodies is shown under higher magnification in Fig. 3. The large vacuoles may be seen distinctly. In these, in the fresh preparation, the Borrel bodies could be seen in rapid Brownian motion. Fowl-pox inclusions, therefore, are composed of a semipermeable ground substance in which the Borrel bodies are embedded. This ground substance is thought to be a lipoproteid in composition.<sup>8</sup>

While working with inclusion bodies which had become swollen in distilled water, it was observed that if the water be allowed to dry, the inclusions, at the moment the water leaves their surface, will be torn into fragments with an abruptness almost explosive in character. The breaking up of the inclusions in this fashion we presume to be due to the force of surface tension, which is relatively enormous when acting upon such minute structures. This method of tearing apart inclusion bodies was found to be ideal for demonstrating the constituent parts of the bodies. Single bodies were isolated and washed, using a capillary pipette and the Chambers microdissection

## THE RELATION OF THE VIRUS OF FOWL-POX TO THE SPECIFIC CELLULAR INCLUSIONS OF THE DISEASE \*

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The nature of cellular inclusions in virus diseases has been for years the subject of controversy. When first observed in fowl-pox, for example, the inclusions were thought to be protozoa which caused the lesion in which they appeared.<sup>1</sup> With the first experiments on filtration, however, any idea that the entire inclusion body represented a single protozoan parasite had to be abandoned on account of the obvious impossibility of any such structure passing intact through the fine pores of a filter.

Some of the more recent workers have not only abandoned the protozoan hypothesis, but have swung to the other extreme of relegating the inclusion bodies to the class of cellular degeneration products.<sup>2,3</sup> That the inclusion bodies of fowl-pox, at least, are indeed something other than mere products of cellular degeneration was demonstrated in a recent publication from this laboratory.<sup>4</sup> It was shown that upon subjecting a fowl-pox lesion to tryptic digestion the inclusion bodies resist the action of the trypsin, while the epithelial cells of the lesion are completely destroyed. The inclusion bodies, thus liberated from their host cells, could then be washed in sterile saline until the fluid immediately surrounding them was innocuous, while a single inclusion body, when inoculated into the feather follicle of a normal chick, produced a characteristic fowl-pox lesion. This demonstration of the infective nature of the inclusion bodies of fowl-pox gives rise to questions concerning the make-up of the inclusions, and especially the question as to what portion or portions of the inclusions, are responsible for the transmission of the disease.

### THE COMPONENT PARTS OF THE INCLUSION BODIES

Since the observations of Borrel <sup>5</sup> in 1904, it has been known that smears of the fowl-pox lesion show innumerable minute, coccoid

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† The initial work on this paper was done while a Fellow in Medicine of the National Research Council.

## THE INOCULATION OF SMALL PORTIONS OF INCLUSION BODIES

Following our successful experience with the inoculation of isolated and washed inclusion bodies in chicken-feather follicles,<sup>4</sup> numerous attempts were made to disperse the individual inclusion bodies in some fashion which would allow the inoculation of their fractional parts. On account of the extreme tenuousness of the lipoproteid material in the bodies, all of our efforts in this direction were fruitless until the method just described of allowing the bodies to dry out of distilled water was devised. With this method at hand it seemed that portions of the inclusion body smear might readily be scraped up for purposes of inoculation. Accordingly, single inclusion bodies which had been freed of cellular material by tryptic digestion, were isolated, using a capillary pipette in the Chambers microdissection apparatus. The single inclusion was then transferred to a pool of sterile distilled water on a large-sized coverslip and thoroughly washed by sucking up the major portion of the distilled water in a pipette and then replenishing the pool, this process being repeated three times. The distilled water was next sucked off completely and the inclusion body allowed to break up. Then the coverslip was inverted over the moist chamber of the microdissection apparatus. To make an inoculation from the inclusion body smear a 4 mm. solid glass rod, which had been drawn to a fine point, was clamped in the Chambers apparatus and forced across the smear. A resulting faint scratch could usually be seen in the substance of the smear along with a minute accumulation of material on the glass point. The tip of this glass point, carrying its small portion of the smear, was carefully inserted into the defeathered follicle of a normal chick and then broken off, to be left, glass and all, in the follicle. As many as ten scratches across the smear of a single inclusion body have been made in this fashion, using a fresh, sterile, glass point for each scratch and inoculation. In each instance all of the scratch inoculations from a given inclusion were made into the follicles of a single chicken. After the completion of the inoculations the coverslip was removed from the moist chamber, stained and mounted and kept as a permanent record of the experiment (Fig. 5).

The experiment has been repeated with seventeen different inclusion body smears. In all, 135 inoculations have been made, fifty-two of which resulted in characteristic fowl-pox lesions. The largest

apparatus. Upon allowing such a body to break up, a smear was obtained which showed the individual Borrel bodies and in which one could know with certainty that there were included only the Borrel bodies belonging to that particular inclusion. Such a smear of a single inclusion body is shown in Fig. 4. The Borrel bodies are the minute round structures appearing sometimes singly, sometimes in diploid form, and again in chains or masses. Under higher magnification the black areas in this photograph can be resolved into closely grouped masses of Borrel bodies embedded in the ground substance of the inclusion.

With the stained preparations of individual inclusion body smears it has been possible to get a fairly accurate idea of their enormous content of Borrel bodies. Actual counts have been made from photographic prints in which the Borrel bodies were magnified 1000 diameters. In each instance one thousand adjacent Borrel bodies were counted and from that count the total number in the smear was estimated. Using this method six thousand Borrel bodies were found in one inclusion, in another twenty thousand, and in a third ten thousand. All of these estimates were conservative and it is believed that a more accurate count would have revealed more, rather than fewer, Borrel bodies.

In order to get an idea of the relative size of the Borrel bodies, hemolytic streptococci from a culture were introduced in a preparation of inclusion bodies in distilled water. A portion of the resulting smear is shown in Fig. 6. The streptococci, on account of their greater size, are in a focal plane slightly different from that of the Borrel bodies. For this reason the streptococci are in the sharpest focus in the lower and right-hand portion of the picture where the Borrel bodies are out of focus.

The stain used in this preparation was Morosow's modification of the Fontana-Tribondeau method<sup>9</sup> and consisted essentially of a mordant of tannin followed by a silver nitrate solution. On account of the mordant the streptococci in Fig. 6 appear much larger than streptococci from the same culture stained by Gram's method. Presumably the Borrel bodies appear similarly enlarged, due to the mordant. By Morosow's method the streptococci and Borrel bodies stain a deep brown or black. The photograph was taken using a blue light.



were negative. Evidently any fluid from the final wash pool which might have adhered to the glass points was non-infectious. Furthermore, the control inoculations demonstrate that the breaking off of the glass points in chickens' follicles does not in itself produce a foreign body reaction in any way suggestive of the fowl-pox lesion.

### DISCUSSION

In the technique of these experiments too many variables are involved to permit a positive statement as to the reason for the great variation in the number of "takes" from a single inclusion body. A cursory examination of the table indicates that in general, the percentage of "takes" was much lower where ten inoculations were attempted from a single smear than where fewer inoculations were made. The element of fatigue on the part of the experimentalist may enter here, for the process of washing and inoculating is a tedious one and it is difficult to introduce one of the fine glass points into the opening of a feather follicle without accidentally brushing it against the outside of the follicle. This latter accident might well brush the infectious material from the point.

With the inoculations WH107 and WH108 a slightly different technique was employed. In "107" the glass points had been dipped in sterile gelatine preceding the scratching process, in the hope that the gelatin might cause the adherence of more infectious material to the point. In "108" hollow points drawn from glass tubing were used instead of points drawn from solid glass. On account of their ineffectiveness these technical variations were abandoned following a single trial of each, and the solid glass points were used in all the other inoculations.

A further factor which was apparently of some importance in the inoculations was the "age" of the inclusion bodies used. In general, better results were obtained with an inclusion from a seven- to ten-day fowl-pox lesion than with one from a lesion two weeks old or older.

The chief significance of the present series of experiments, lies, we believe, in the demonstration of the infectious nature of material from seventeen different inclusion bodies which had been digested and washed free of all particles of cellular material and then allowed to disintegrate so as to liberate their component Borrel bodies. Also of

number of successful inoculations from a single smear was six, obtained in two instances, while from each of three smears only a single "take" was obtained.

The determination of a "take" was based upon the development of the characteristic firm, white swelling of the follicle (Fig. 7). In case any doubt existed, the follicle was removed and digested in trypsin, a positive result then being based upon the demonstration of inclusion bodies.

TABLE I

*Showing Results of Scratch Inoculations from the Smears of Single Inclusion Bodies*

Number of inoculated chicken	Number of scratch inoculations from the inclusion body smear	Number of positive results	Control inoculations (all negative)
WH 89 .....	6	3	<i>No control</i>
WH 92 .....	3	2	<i>No control</i>
WH 93 .....	8	5	<i>No control</i>
WH 104 .....	10	4	10
WH 105 .....	10	2	10
WH 106 .....	10	3	10
WH 107 .....	10	1	10
WH 108 .....	10	1	10
WH 109 .....	10	2	10
WH 110 .....	10	2	10
WH 111 .....	10	4	10
WH 112 .....	6	6	6
WH 113 .....	6	2	6
WH 114 .....	4	1	4
WH 115 .....	8	6	8
WH 116 .....	6	5	6
WH 117 .....	8	3	8
Total .....	135	52	118

As indicated in the above table, no control inoculations were made in the first three experiments. In the fourteen subsequent experiments control inoculations were made by running sterile glass points through the pool of distilled water used in the final washing of the inclusion body. The points were run close to the body, but care was taken that they should not actually touch it. The tips of these glass points were then broken off in feather follicles on the opposite breast of the same chicken that was to be used for inoculations from the inclusion body smear (Fig. 7). All 118 of these control inoculations

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## DESCRIPTION OF PLATES

### PLATE 129

- FIG. 1. Inclusion bodies in saline.  $\times 180$ .
- FIG. 2. Same inclusion bodies thirty minutes after distilled water had been substituted for the saline.  $\times 180$ .
- FIG. 3. Inclusion body swollen in distilled water.  $\times 1000$ .
- FIG. 4. Smear of single inclusion body broken up by the surface tension of a drying film of water. Borrel bodies are seen as the minute coccoid structures. Morosow's stain. Photomicrograph taken with blue light.  $\times 510$ .
- FIG. 5. Inclusion body smear from which the six "scratch" inoculations of WH112 were made. The marks of two of the scratches are plainly visible. Morosow's stain. Photomicrograph taken with blue light.  $\times 360$ .

importance is the demonstration of the capacity of such an inclusion body to furnish infectious material for as many as six positive inoculations. The limits of this subdivision of an inclusion body we feel has by no means been reached.

It is impossible to say from these experiments that one specific portion of the inclusion body smear alone was responsible for the successful inoculations. Obviously some of the ground substance of the body is picked up on the glass point as well as the Borrel bodies. However, the lipid component of this ground substance has been demonstrated to be non-infectious by inoculation of both alcoholic and ether extracts of the inclusion bodies, and we have not as yet been able to isolate any protein component of the inclusions distinct and apart from the Borrel bodies. These facts, together with the enormous numbers of Borrel bodies in a single inclusion and their perfect uniformity in size and shape, lead us to think that in our various experiments the Borrel bodies represent the important part of the inoculum — the actual virus of fowl-pox.

### SUMMARY

1. Inclusion bodies of fowl-pox may be broken up by using the surface tension of a drying film of water. The stained smear of an inclusion body thus disrupted has been shown to contain as many as 20,000 Borrel bodies — minute coccoid structures uniform in size and shape.

2. Multiple inoculations have been made from the smears of isolated and ruptured inclusion bodies with as many as six inoculations from a single inclusion resulting successfully. Control inoculations were all negative.

3. The lipid component of the inclusion bodies is non-infectious. Aside from this lipid, the Borrel bodies form the major constituent of the inclusions and are judged to represent the actual virus of fowl-pox.

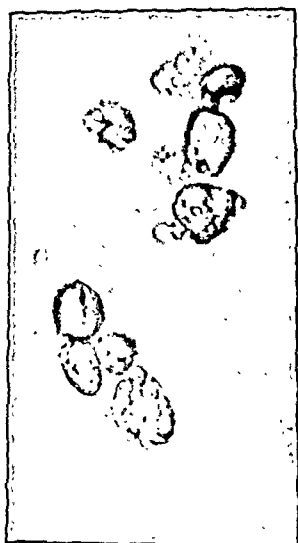
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PLATE 130

FIG. 6. Smear showing comparative size of Borrel bodies and hemolytic streptococci. Morosow's stain. Photomicrograph taken with blue light.  $\times 2200$ .

FIG. 7. Skin from breast of chicken showing at left three follicles ten days after each had been inoculated with material scratched up from a single inclusion body smear. Control inoculations at right.  $\times 0.8$



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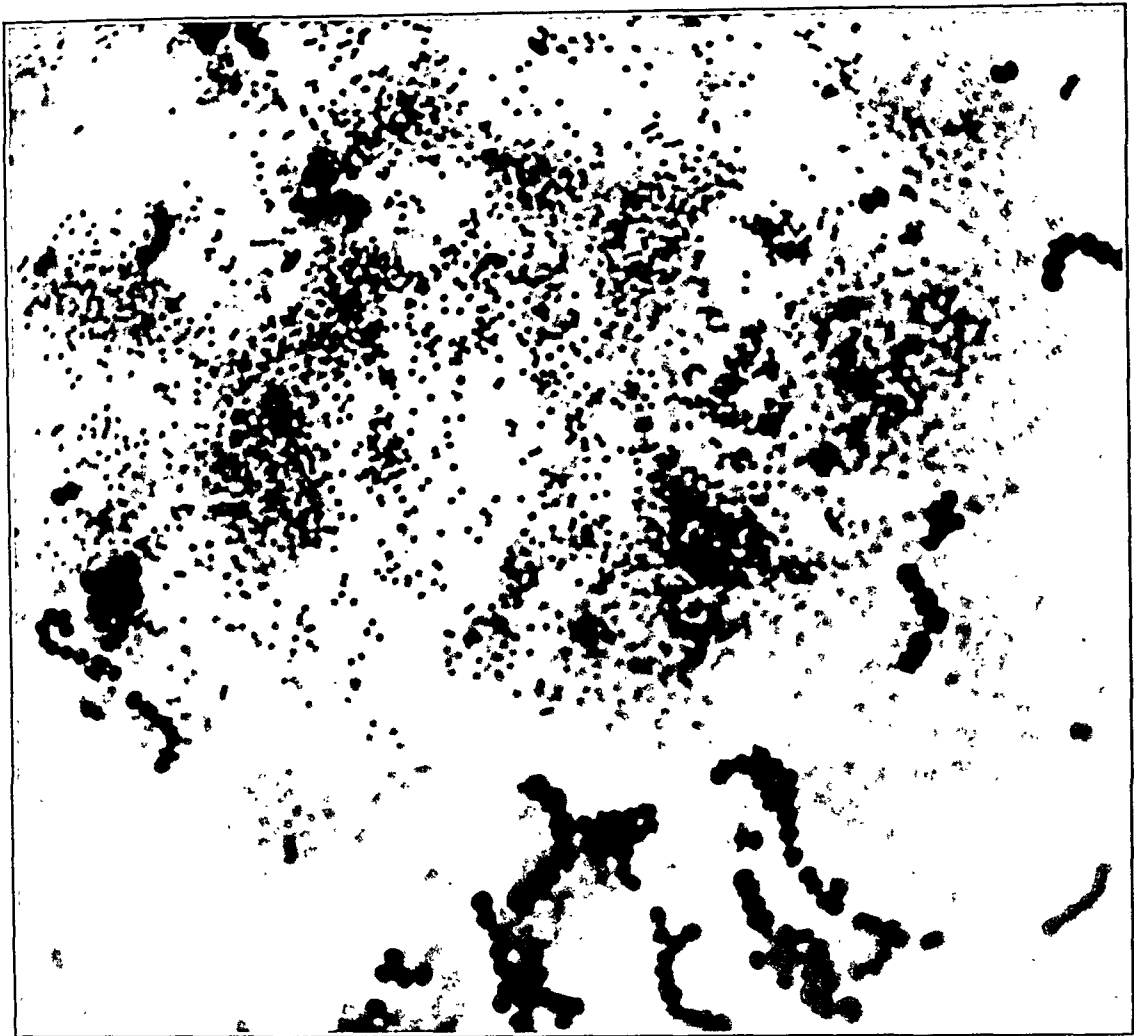


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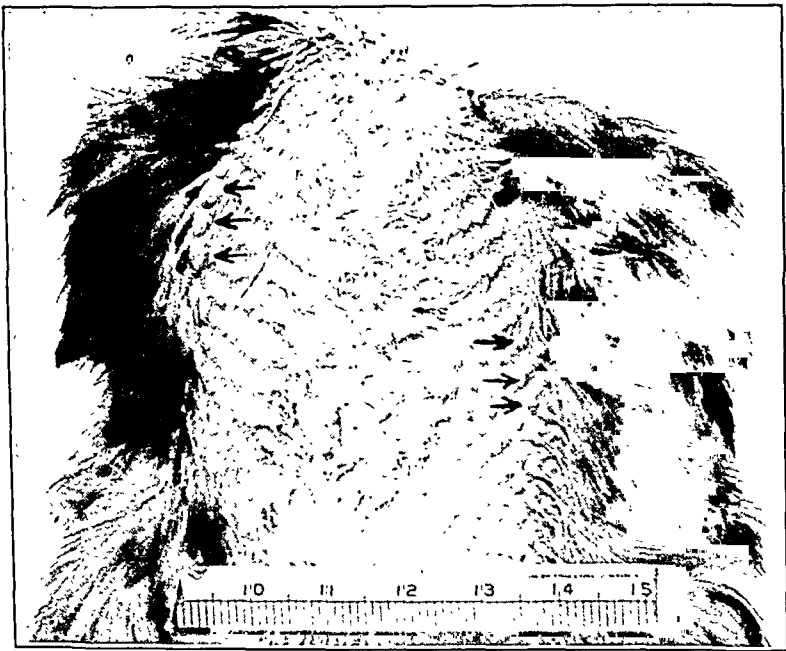


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man was a native of Bihar and had been working as a mason for two and one-half years. However, he had first noticed the growth in his nose three years before while working in a hide store. He was seen by O'Kinealy in Calcutta in 1894. There had been two previous partial removals of an obstructing polypoid mass and when the patient was last seen there was again evidence of recurrence following a third operation. Upon examination he presented a small vascular pedunculated tumor about the size and shape of a large pea, freely movable, painless and having the appearance of a papilloma. It was attached by a short pedicle to the mucous membrane at the anterior and upper part of the cartilaginous septum, was friable and bled freely during the course of its removal. The pathological description was furnished by Major J. C. Vaughan. In this report O'Kinealy stated that he had reason to believe that similar cases had been observed in Calcutta, though he did not think that they had been published.

A few sections from the case described by O'Kinealy were reworked by Minchin and Fantham,<sup>4</sup> who, in 1905, gave a further description of the parasite, differing in some particulars from that of O'Kinealy. At this time they proposed the name *Rhinosporidium kinealyi* for the etiological agent.

In 1906 Beattie<sup>5</sup> described the material from a nasal polypus which had been supplied him by Dr. Nair of Madras, who stated that he had had four similar cases. All of these were from the state of Cochin on the west coast of India. This material was seen by Minchin and was pronounced similar in every respect to that from which he had described *Rhinosporidium kinealyi*. In one of his papers Beattie refers to the cysts produced as being 6 to 8 mm. in diameter. These measurements were obviously incorrect and Ashworth<sup>6</sup> has found no cysts exceeding 0.2 to 0.3 mm. in diameter in the very slides which Beattie used. In the following year Beattie<sup>7</sup> recorded the occurrence of the same organism in aural polypi, but without a detailed case report.

The first American case was described by J. Wright<sup>8</sup> in 1907. Wright had received this material from Dr. E. C. Ellett of Memphis, Tennessee, in 1903, but it was not diagnosed until after the appearance of the papers by O'Kinealy, Minchin and Fantham, and Beattie. The patient was a young farmer, 20 years old, who had never been away from the neighborhood of Memphis. He had sub-

RHINOSPORIDIUM SEEBERI: PATHOLOGICAL HISTOLOGY  
AND REPORT OF THE THIRD CASE FROM  
THE UNITED STATES \*

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The organism now known as *Rhinosporidium seeberi* has been described from three geographical regions, far distant one from another, Argentina, India and the United States. The instance of infection with it which is reported here is believed to be but the third from this country. Likewise but three cases of this disease have been described from Argentina, as far as we can learn. In India, however, it has been recognized many times since its first description from that country in 1903. There are in the literature at least twenty-five case reports from India and Ceylon, several of them dealing with the occurrence of this infection in anatomical locations other than the nose.

The first description of this parasite was published in a thesis from Buenos Aires in 1900, by Guillermo Seeber.<sup>1</sup> We have been unable to obtain this in its original form but a quite complete abstract can be found in the historical section of Ashworth's<sup>2</sup> paper on Rhinosporidium. The patient was an agricultural laborer, 19 years of age. Although born in Italy, this young man had lived in Argentina for eighteen years. He presented an obstructing polypoid growth of the left nasal fossa projecting through the external orifice. Upon this material, and upon that obtained at a second operation necessitated by recurrence, Seeber's description of the organism was based. In an appendix, note is made of a still earlier, but unpublished case, observed in 1892 by Professor Malbran of Buenos Aires. In 1912 Seeber republished a summary of his thesis and at that time referred to still another case found by Malbran, thus bringing the total to three for Argentina. It was this republication which brought the unrecognized priority of Seeber to the attention of other investigators in this field.

In 1903 O'Kinealy<sup>3</sup> described as a local psorospermiosis a growth from the left nostril of a male Mohammedan, 22 years of age. This

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conjunctiva. In a second paper <sup>16</sup> the same author described the treatment and apparent cure of one of his conjunctival cases by the use of 2 per cent tartar emetic dropped into the eye three times a day over a period of three months, and also recorded a new case in which there were large polypi upon the inferior and middle turbinates. In this patient the lacrymal sac, the size of a filbert, was found upon extirpation to consist of a fibrous wall with polypoid projections completely filling the interior. In a subsequent report, with Tirumurti, Wright <sup>17</sup> referred to the occurrence of the same parasite in a papillomatous growth of the uvula.

The entire field of information concerning *Rhinosporidium* was very completely and critically reworked by Ashworth <sup>2</sup> in 1922. By him the designation *Rhinosporidium seeberi* was established as previously described and an extremely thorough and systematic morphological description made. He corrected numerous mistakes in the earlier accounts and worked out, on the basis of morphology alone, an acceptable life history. We find the organism in our own case to be in every significant respect in accord with Ashworth's description.

The second North American case was described by Drs. Mary C. Lincoln and Stella M. Gardner <sup>18</sup> in 1929. The patient was a man, 40 years of age, who was born near Carthage, Illinois, and who had lived there until he was about 17 years old. He then spent three years in Chicago and one year in Oklahoma. In 1925 he was in Florida for nine months. He had never been outside of the United States. Thirty years before he had had an operation performed on his nose and at that time the septum was perforated. There was no further difficulty for twenty-two years until he commenced to have a discharge and occasional bleeding. Eight years later the polypoid tumor from which the diagnosis was made was removed by Dr. M. C. Van de Venter of Keokuk, Iowa. The specimen had the appearance, grossly, of an ordinary nasal polypus. Microscopic sections showed the organisms in large numbers and in practically all stages. Very good photomicrographs are used to illustrate the more important morphological features of the organism. These leave no doubt that the parasite has the same structural characteristics as did those described by Seeber from South America and by the various workers upon the material coming from India and from Ceylon.

mitted to a series of operations beginning with the removal of a growth from the lower anterior part of the right septum in 1897. The second operation, in 1898, was upon the anterior portion of the lower right turbinate. Still another operation was performed in March, 1902, and the removal of the specimen examined was accomplished by Ellett in December of the same year. He found three areas involved, the old sites on the septum and turbinate and a new area on the septum. There was no evidence of recurrence two years later.

Growths produced by this same organism were described from the conjunctiva by Ingram<sup>9</sup> in 1910, and by Elliot and Ingram,<sup>10</sup> and by Kirkpatrick<sup>11</sup> in 1912, a total of three cases. The anatomical distribution of the lesion resulting from this infection was extended further by the description by Ingram in his earlier papers of a growth of six years duration involving the entire glans penis of a patient 45 years old.

In 1912 Seeber published a summary of his earlier thesis which was thus brought to the general knowledge of protozoölogists. His priority was established by investigations instituted by Ashworth<sup>2</sup> for it was found that the name *Coccidium seeberi* had been assigned to this organism in Belou's *Parasitologia Animal* in 1903. Accordingly Ashworth emended the name to *Rhinosporidium seeberi*, which has been generally accepted since his monograph appeared in 1922.

Tirumurti<sup>12</sup> collected, in 1914, fifteen instances of Rhinosporidium infection in each of which tissue specimens had been examined in the pathological laboratory of the Madras Medical College. Several of these had been reported previously by other authors, but in addition to these, he described eleven new cases of nasal and nasopharyngeal involvement. Further case reports have followed. From the General Hospital of Colombo, Ceylon, Chelliah,<sup>13</sup> in 1918, described three additional examples from the nose in males, aged 17, 19 and 58 years respectively. Two of his patients had never been in India and one had never been out of Ceylon. Two years before Kirkpatrick<sup>14</sup> had reported for the first time the occurrence of Rhinosporidium infection in the lacrymal sac. In 1922 R. E. Wright<sup>15</sup> recorded three conjunctival cases which had been seen within a period of three months in the Civil Orphan Asylum in Madras. The patients were Eurasian boys whose ages were 13, 14 and 14 years of age. One of these had a growth on both the upper and the lower palpebral